POTENTIAL APPLICATION OF RECOMBINANT DNA AND GENETICS ON AGRICULTURAL SCIENCES

HEARINGS

BEFORE THE

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INVESTIGATIONS AND OVERSIGHT

OF THE

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POTENTIAL APPLICATION OF RECOMBINANT DNA AND GENETICS ON AGRICULTURAL SCIENCES

WEDNESDAY, JUNE 9, 1982

House of Representatives,
Committee on Science and Technology,
Subcommittee on Investigations and Oversight,
Washington, D.C.

The subcommittee met, pursuant to notice, at 9:40 a.m., in room 2325, Rayburn House Office Building, Hon. Albert Gore, Jr. (chairman of the subcommittee), presiding.

Present: Representatives Gore and Volkmer.

Mr. Gore. The subcommittee will come to order. I would like to welcome all of our guests and our witnesses this morning.

This subcommittee has been concerned in prior hearings with the ethical and legal implications of recombinant DNA research, and while these examinations are certainly not at an end, this morning we are moving into a new avenue of inquiry, namely, what are the potential applications of recombinant DNA and genetic engineering techniques and what is the likely impact? This is the first of two hearings in this regard. Later this summer we will have a hearing on the impact to be expected in the livestock industry and animal husbandry. This morning's hearing will be focused on plants.

As I am sure everyone in the room is aware, agriculture has been held up by many observers as the first sector where we can expect to see concrete results of this celebrated area of biology. Combining biotechnology with agriculture has conjured up miraculous images: alfalfa that grows in the west Texas desert; wheat with more protein; and cash crops that do not need pesticides. All of these are among the many possibilities that have come from our efforts to build a better biological mousetrap.

However, while this research is certainly exciting and heady stuff indeed, it is important that Congress, and especially the Science and Technology Committee, have a realistic sense of what to expect from DNA applications and, just as importantly, when to expect it. Therefore, one of our prime efforts today will be to examine the varying assessments of the state-of-the-art and leadtimes in this new corridor of science.

While some observers are quick to tell us about the possibilities, others involved in agricultural research see a far more limited role for genetic engineering. For that same reason, while some major and midsized corporations are making substantial if not remarkable investments in biotechnology, other concerns such as some seed companies have far more modest scientific and financial strategies.

It is also part of our inquiry this morning to see how agriculture is going to reap the potential benefits of recombinant DNA techniques in the face of a severe shortage in the necessary scientific manpower and rather miniscule Federal contributions to the various ongoing and proposed avenues of research. Lastly, it is always our responsibility to insure that the appropriate role for the Federal Government in any R&D effort is being fulfilled, which in this case is the province of the Agricultural Research Service.

We thank all of our witnesses in advance for their time and effort. I would like to ask unanimous consent that the record be held open at this point for the inclusion of a statement by the ranking minority member, Mr. Walker, and others among my colleagues who I know have an interest in this area.

[Opening statement of Mr. Walker follows:]

OPENING REMARKS OF ROBERT S. WALKER - JUNE 9, 1982

MR. CHAIRMAN, OUR HEARING TODAY OF PARTICULAR IMPORTANCE TO ME BECAUSE IT IS AT THE VERY CENTER OF THE CONTROVERSY SURROUNDING THE FUTURE OF AGRICULTURE IN THIS COUNTRY.

I TAKE GREAT PRIDE IN REPRESENTING THE FINEST AGRICULTURAL AREA IN THE UNITED STATES. WE IN LANCASTER COUNTY ARE THE HEART OF THE PENNSYLVANIA DUTCH FARMLAND. AND WE KNOW THAT OUR FUTURE, JUST AS OUR PAST, LIES DEEPLY ROOTED IN THE FERTILE SOIL OF PENNSYLVANIA.

DESPITE THE FACT THAT MOST PEOPLE ENVISION GREAT FACTORIES AND TECHNOLOGICAL WONDERS WHEN THE THINK OF THE PROPER PLACE OF THE UNITED STATES IN WORLD TRADE -- THE TRUTH IS THAT OUR ONE GREAT ECONOMIC ADVANTAGE OVER THE REST OF THE WORLD LIES IN AGRICULTURE. WE ARE THE GREAT FOODBASKET OF MANKIND.

BUT THERE ARE NUMEROUS FORCES THAT ARE AT WORK TO REDUCE - OR EVEN END THAT GREAT ADVANTAGE. ECONOMIC AND TAX POLICIES PLACE TREMENDOUS STRAINS ON THE INDIVIDUAL FARMER. WE HAVE WORKED, AND WILL CONTINUE TO WORK TO SEE THAT THE FARMER IS TREATED WITH EQUITY, AND THAT OUR TAX POLICIES DO NOT MAKE IT IMPOSSIBLE FOR FARMERS TO PASS ON THEIR FARMS TO NEW GENERATIONS.

THE EROSION OF VALUABLE FARM SOIL HAS REACHED GIGANTIC PROPORTIONS AND MUST BE BROUGHT UNDER CONTROL. NEW METHODS OF TERRACING HAVE BEEN DEVELOPED AND ARE BEING IMPLEMENTED ALL ACROSS AMERICA WITH GOVERNMENT HELP IN SOME CASES.

THE GOVERNMENT IS FINALLY STARTING TO ADDRESS THE PROBLEM OF URBAN SPRAWL OVER VALUABLE FARMLAND. I AM PROUD TO SAY THAT UNDER MY URGING THE ENVIRONMENTAL PROTECTION AGENCY SEVERAL YEARS AGO ADOPTED A POLICY OF AFFIRMATIVELY PROTECTING FARMLANDS.

But there is still a great threat to agriculture as we fully discover the problems associated with the massive use of chemicals over a period of decades. In Lancaster County we do not have the same problems because the Pennsylvania Dutch have always been frugal in the chemicals they have applied, using as little as possible, and biodegradeable products where they were available. For generations they have renewed the earth with organic products just as was done in biblical times. But, in other parts of the country the residual wastes of a generation of heavy chemical use remain like a ticking time bomb -- waiting and still dangerous.

As we read about bio-technology in the popular press there is always the promise that this may be the key that will unlock a new corncopia. Crops that will be disease and ensect free. Crops that will be damage resistant in times of srought. Crops that will require less work on the part of the farmer, and which will will be highly energy efficient because they will not need synthetic fertilizers.

Some of these promises are already on the horizion. Others are just beyond man's reach at the present. And some may never be realized.

There are a number of serious questions which must be addressed before we enter this brave new world in which man designs plants to meet his needs. We, in policy making positions in the government need to deside exactly what the role of government can, or even should be in this area. We need to determine if there should be institutional encouragements to research in this area, and if governmental action is needed either to encourage

THAT RESEARCH, OR TO INDUCE YOUNG STUDENTS TO MOVE INTO THIS NEW AREA. THESE ARE QUESTIONS WE WILL HAVE AN OPPORTUNITY TO DISCUSS TODAY IN THE COURSE OF THIS HEARING.

THIS WEEK WE ARE IN THE MIDST OF A GREAT BATTLE HERE IN THE CONGRESS. THIS WEEK MAY MARK ONE OF THE GREAT BENCH MARKS IN OUR GOVERNMENT. PRESIDENT REAGAN CAME TO WASHINGTON WITH A GREAT MANDATE FROM THE PEOPLE OF AMERICA WHO HEARD HIS PROMISE TO CHANGE THE COURSE OF GOVERNMENT. THE BATTLE OF THE BUDGET BEING WAGED IN THE HALLS OF CONGRESS THIS WEEK MAY WELL DETERMINE IF HE WILL BE SUCCESSFUL.

BUT, AT THE SAME TIME THAT WE ARE CLOSING OLD, AND UNSUCCESSFUL PROGRAMS, WE MUST ALSO BE CAREFUL THAT WE CONSTANTLY REALIZE THAT THERE ARE PROPER PLACES FOR GOVERNMENT ACTION, AND GOVERNMENT CONCERN. THIS MAY WELL BE ONE OF THOSE AREAS. LONG TERM RESEARCH AND DEVELOPMENT THAT MIGHT CONTINUE OUR AGRICULTURAL ADVANTAGE WELL INTO THE NEXT CENTURY IS AND AREA THAT BEARS THE CLOSEST SCRUITENT. I AM LOOKING FORWARD TO HEARING THE WITNESSES BEFORE US TODAY.

Mr. GORE. Our first panel of witnesses consists of Dr. Winston Brill of the University of Wisconsin, and also director of research for the Cetus-Madison Corp.; Dr. Ralph Mott from the Department of Botany at North Carolina State University in Raleigh; and Dr. Aloysius Wood, dean of the College of Agriculture at the University of Florida in Gainesville.

Gentlemen, if the three of you would make your way to the witness table and join us, I would like to welcome all three of you. Dr. Wood, I want to give you a special welcome on behalf of the chairman of the full committee——

Mr. WOOD. Thank you.

Mr. GORE [continuing]. And the staff director, Dr. Hanson, both of whom count you as a close friend. To all of our witnesses on this panel, let me welcome you and say that the prepared text that you have given to us will be inserted in full in the record, so if you slip over a word or something, don't worry about it. If you care to summarize any portion of it, that is fine, too.

Let me invite you, Dr. Brill, to lead off. We are honored to have you with us. You are held in high esteem in this area of science and we are delighted to hear from you this morning. Please proceed.

STATEMENT OF DR. WINSTON J. BRILL, VILAS RESEARCH PROFESSOR OF BACTERIOLOGY, UNIVERSITY OF WISCONSIN, AND DIRECTOR OF RESEARCH, CETUS MADISON CORP.

Dr. BRILL. I would like to make several points in my 10-minute talk and most of them are amplified in the statement that I handed in.

Let me first talk about the state of the art. U.S. agriculture is very successful and has been very successful over the decades for several reasons, one, its excellent breeding programs; another, the fact that it could supply itself with rather cheap nitrogen fertilizer; and, third, that the farmers used quite a bit of chemicals to antagonize pests and weeds.

Things are changing a bit now. The breeding programs seem to be leveling off with respect to their ability to increase crop yields. Fertilizer costs are increasing tremendously and are expected to increase in the future. Nitrogen fertilizer, coming from a very energy intensive technology, is a major cost in agriculture now. Also, the use of chemicals has side effects, causing problems on the plants, causing problems for the farmer, and causing problems for the consumer.

Biotechnology has not made impacts into any of these yet but has a promise for helping these and other areas of agriculture in the future. Now plant biotechnology is far behind bacterial and animal—including human—biotechnology. I think that is very important. However, the basic plant sciences are now, just in the past few years, beginning to buzz. They have an excitement of their own, an excitement that has not been seen in this area in the past. One is seeing industries paying more attention to basic sciences and also universities now paying a little bit more attention to the applied sciences.

There are many, many possible applications. The ones that I think are going to be achieved in the short term are ones where the technologies are based on technologies stolen from the animal and bacterial sciences, so they include things such as diagnostics. There is a lot of biotechnology being applied in the health-care industry for diagnosing certain diseases and certain genetic problems, these diagnostic techniques can be and are being applied now. They are being examined to look at plant problems, virus disease, bacterial or fungal infections, diagnosing diseases before the farmer sees any adverse effect on his plants and then he could make an early judgment whether to spray or not. That is relatively easy to do.

A little bit more complicated, I think, in the short term are diagnostics as applied to looking at the plant's genes rather than looking at what the plant looks like. That means that one could possibly tell at a very early stage in the plant growth cycle whether that plant has resistance to a disease, whether that plant will yield whatever one is interested in, whether that plant will have a certain color flower or whatever. There are techniques now being used to try to diagnose or try to predict what a plant will look at in its mature state, and that will be extraordinarily useful to the plant breeder.

Another relatively short-term set of applications are those that involve bacteria themselves. Most of biotechnology has been performed with bacteria, and there are bacteria that have been useful in agriculture for a long time. An example are nitrogen-fixing bacteria, which happens to be my area of expertise. Nitrogen-fixing bacteria will allow certain plants like the legumes, alfalfa, soybean, bean, peanuts, and clover to grow without the requirement of fertilizer. Therefore, biotechnology has some promise, in fact some actual success, in making these nitrogen-fixing bacteria perform even better for the plant. There has been some success in raising soybean yields by fiddling around with the bacteria. We certainly know how to easily manipulate bacteria.

Other things are to try to get bacteria to hang on roots of plants, to produce growth harmones that the plant might like. Other ideas include using bacteria to decrease frost damage. There has been success in that area. There are some breakthroughs in being able to induce resistance of a plant to a certain bacterial or fungal pathogen by adding chemicals from the pathogen to the plant, so it is almost like our immune system. If you see a problem or you are afraid of a problem, you spray the plant with a certain chemical which is not generally toxic and that chemical will allow the plant to become resistant to that disease.

In the long term—and I am not sure how to define long term because surprises are occurring all the time, and I think that generally the scientist 2 years ago probably did not predict that we would be as advanced as we are today—so what I see in the long term includes the targeting of mutations. In other words, you want the plant to produce more of this protein, more of this enzyme, and one can very cleverly say, "I will modify this piece of DNA," and it will be done.

A little bit more exotic is genetic engineering of plants. Nobody has yet genetically engineered a plant to do anything, so nobody has yet gotten a plant to produce something that it ordinarily does not produce, but progress is being made, and I think it is just a matter of time until one can genetically engineer plants.

One use of genetic engineering will be to aid in plant breeding. For instance, if there is a tropical corn that has a gene of interest or several genes of interest and one wants to breed those genes into a modern Midwestern corn, the way it is done now is through a very extensive breeding program where one has to backcross to eliminate some of the undesirable genes that came in from the tropical plant, whereas through genetic engineering one could be smart enough to pick out the 5 genes, 10 genes, 1 gene of interest and insert those genes into the Midwestern plant without worrying about detrimental genes.

Then one could possibly in the future—and it definitely will happen in the future—put foreign genes, genes from other plants, into plants of interest.

Comments on the commercial potential. The field is really just beginning and there needs to be a tremendous amount of legwork to get some of the basic concepts down if we are going to take advantage of this technology in the future. I am very enthusiastic about the commercial potential of this and that enthusiasm mainly comes from my less than a year involvement, half-time, in a biotechnology company. In that time, we have come up with two projects that really came out of side projects, that came out of an interdisciplinary effort between several scientists, that we think will make profits for the company within 2 to 3 years.

Therefore, the opportunities are wide open and I think that what we need are dedicated teams and resources, money, to get into these areas, and I think we are guaranteed to make major advances.

Since I have two jobs, one in the university and one in industry, let me just make a comment that I think there are many types of university-industry relationships that can be beneficial to the industry, the university, and the public in general. There is certainly potential for mischief and for troubles but I do not see that troubles have to be the rule. I can comment on that later.

Then the last comment is about manpower. We have to somehow overcome the barrier of past neglect. The plant sciences have not at all attracted major interest. There are certainly exceptions—but generally major interest and major funding has been lacking. Now all of a sudden there is a big interest in basic plant sciences. Where are all the people? These are only a few excellent laboratories. There is a large number of people willing to accept money to do plant work. The amount of money put into training of sophisticated basic plant researchers is really trivial when you compare that to the health sciences or almost any other sciences where there has been quite a bit of interest.

There are no training programs, no postdoctoral programs, and that is a barrier we have to overcome. I think it is going to be very important not just to throw money in but to think about how to spend the money in order to get the most out of it for the future.

Thank you.

[The prepared statement of Dr. Brill follows:]

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SOME THOUGHTS REGARDING AGRICULTURAL BIOTECHNOLOGY

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Statement for Committee on Science and Technology, Subcommittee on Investigations and Oversight. Testimony on June 9, 1982.

These last few years have been extremely exciting for researchers studying plant molecular biology, biochemistry and other basic plant science areas with a genetic focus. The attention and funding given to basic plant scientists over the past four years has increased dramatically. The press tells us that agricultural biotechnology will help us raise farm productivity and will also yield tremendous profits for private industry. What has created this new environment? Is this all hype or is there some reality to these promises? What problems might we be facing over eagerness generated by the excitement?

The U.S. is an agricultural leader for a variety of reasons--one of them being the excellence, over many decades, of our plant breeding programs. Plant breeders have increased yields, made plants more uniform, and created plants resistant to major pests. The use of fuel-based nitrogen fertilizer allows the farmer to achieve high yields. Chemicals added to plants or to soils decrease competition by weeds and pests such as insects and fungi. Today, however, the previous dramatic rate of yield increase resulting from breeding and proper fertilization seems to be approaching a limit. Fertilizer costs are major expenditures in agriculture due to the increased price of fuel required for fertilizer production. Also, heavy use of nitrogen fertilizer results in run-off problems and pollution of our lakes and streams. Pesticides and herbicides that are added to fields have undesirable side effects (to the farmer, the crop, and the consumer).

Biotechnology has the potential to further increase plant yields, to decrease fertilizer requirement, and to manage weeds and pests without a great dependency on dangerous chemicals. This promise (there have been no actual profitable applications yet) has stimulated large, traditional corporations to hedge their bets on the future by becoming involved with the new biotechnology. At the same time, small new companies have been formed with confidence that the available opportunities, their own innovative spirit and the challenge will enable them to survive and make important contributions even with the background of activity by large corporations. The future is very cloudy, but there seems to be room for many to respond to the challenge of fulfilling the promise of biotechnology.

Until the last four or five years, there was little interest or federal money put into quality basic plant science research. Even though a USDA competitive grants program for basic plant sciences has begun, the funding level is quite low and specific areas of focus have been pre-determined. Compare the massive granting programs in bacterial and animal (including human) basic sciences with the funding level of basic plant sciences. Very few plant research institutes exist, very few plant researchers have been given national honors, few funded training programs are available for plant research, jobs in basic plant sciences existed mainly in the universities. If one wanted to have an exciting future in basic research (e.g. molecular biology or biochemistry), plants were not the way to go. For these and other reasons, our understanding of the basic mechanisms of plant development has lagged far behind that of the development of bacteria and animals. Previously, the more dynamic scientists generally were not concentrated in departments focusing on the plant sciences. Changes are occurring; but, in an economic environment that is slowing down basic research, the changes are coming much too slowly.

The stimulus for current interest in agricultural problems by scientists with the most sophisticated backgrounds and by industries wanting to make profits in agriculture is the imminent success of biotechnology as applied to pharmaceutical problems: insulin, interferon, growth hormones, vaccines, etc. Industry's attraction to the area is obvious. Scientists who normally paid no attention to the potential applications of their research have been made aware of the challenges to bridge the gap between basic research and applications of that research. Previously, there was very little opportunity for a professor to take the "next step" and demonstrate the application potential of a particular discovery. From the other direction, industry generally did not have the insight to foresee the potential application of an undeveloped piece of basic work. This is changing now; hopefully for the long-term benefit of the public.

Due to the present poor state of understanding of basic plant sciences, there are major research investments to be made before most of the applications will be realized. Industry will have to pay for much of this--the federal government has not yet made major financial commitments. There are many approaches being discussed to solve problems in agriculture. No one group can dream of tackling all of these approaches and this is one of the reasons why small companies focusing on several limited objectives feel that they can make important contributions. The research directions in plant sciences are far less clear than many of the pharmaceutical interests. For instance, insulin production by bacteria only required labor and optimization of production. The direction of approach was obvious to scientists. Increasing the protein quality of a grain crop requires many breakthroughs in research--most of them have yet to be made. The plant scientist does not have a logical and clear path to follow because we do not have sufficient basic understanding of plants. Improving plants by new technologies will not be easily attainable, but provides tremendous opportunities for the dedicated and funded.

Advances are being made in plant sciences at an increasing, but far from an ideal, rate. A greater number of creative scientists from a variety of disciplines are beginning to study plant biology and dedicated people are looking for training in this area. Industry and the federal government are supporting national and international meetings in basic plant biology. Industry also is paying for an increasing amount of research (mostly in areas to the company's interest) both in-house and in the university. It is difficult to estimate when the developing technology will help the consumer and become a major profit-making industry, but the rate of basic scientific advances has been greater than predicted only a few years ago by experts in the field. Important results are being obtained with increasing frequency. Similarly, applied successes also should be realized at a rate greater than predicted from yesterdays data base. In my own experience, with less than one year associated with an agricultural biotechnology firm, we have advanced several projects with a very good chance for beneficial and profitable application within the next two years. The most interesting point is that these projects were generated from side observations from other projects, and through interaction of scientists of very diverse backgrounds. We believe that no one else has yet even thought of the approaches we are now using. Other corporations are assumed to be choosing unique approaches for their own projects. Experience such as this gives me confidence that there will be many major contributions--applied and basic--within this decade. Persistence, funding, direction, and talented people are all required for optimized success.

There seem to be immediate possibilities for plant biotechnology to benefit agriculture in developing countries as well as advanced U.S. agriculture. Some people predict that current energy intensive farming practices in the U.S. cannot continue. Our soils are decreasing in fertility and salt is concentrating on valuable farmland. For reasons such as these, U.S. farming of the future may have many more problems in common to those of developing countries than they do now. Therefore, benefits now made possible to less developed nation's farmers may one day help our our farmers. This gives the U.S. selfish as well as magnanimous incentives for helping developing agriculture. Hopefully, this will encourage international cooperation. Agriculture in developing countries now has technologies, plants and traditions with potential benefit to the U.S. An example is the present U.S. interest in the use of ferns to fertilize rice--a centuries-old tradition in Southeast Asia. One concern now is that many of the world's exotic plants are being destroyed by "civilization". These plants have interesting and unusual qualities and could very well be useful sources of new crops as well as for genes for genetic engineering into current crops. Once these plants are gone, this resource will be lost forever. Remember that neither soybean nor alfalfa were native American plants, but our economy now depends on these plants.

Biotechnology has the potential of making major contributions to agriculture. The specifics of these contributions cannot be stated with any kind of certainty. Did researchers working on early experiments with transistors even think about the possibility of a digital watch, medical electronic technology or the office word processor? One of the first uses, in agriculture, of biotechnology probably will be aids to traditional plant breeding. Specific genes can be detected directly in a seedling instead of waiting a full growing season until the characteristics coded by these genes can be tested. Plant cell culture offers many exciting possibilities since it is possible to grow plants from a single cell. This technology has been used with several plants and it is just a matter of time before all major crop plants will be amenable to plant regeneration from single cells. At the single cell stage, genes coding for desirable characteristics can be introduced, one at a time, into plants. This has the advantage over breeding in that the traditional approach usually introduces undesirable characteristics in a cross (along with the desired trait) and these undesired genes can only be removed by a laborious breeding program taking many years to reach completion. Novel ways to introduce specific mutations in genes of interest are being tried. A lot of research is being pursued to provide means for adding foreign genes into plants. Microbes, interacting with plants, also can be manipulated genetically to increase crop yield.

Plants naturally produce many chemicals that are quite valuable--pharmaceuticals, coloring agents, pesticides, flavoring agents, etc. Several groups are placing a great deal of effort into manipulating the plants so that the product of interest is produced in far greater quantities. Another approach takes advantage of cell culture techniques in which plant cells are grown in huge fermenters and the product is concentrated and purified from the fermenter. This approach is similar to that of growing microbes in fermenters for antibiotic production.

The previous lack of interest in plant research is catching up with us in other ways than the dearth of basic available information. The number of plant molecular biology and biochemistry experts in the U.S. is limiting. Most of the well-known professors in this area are now consulting for one or another corporation. Well-trained Ph.D.'s with expertise in agricultural biotechnology are in very high demand. A potential problem that we may be facing is that the new focus in the area is bringing in, not just excellent and dedicated scientists, but also scientists who are merely interested in jumping on the bandwagon. The latter people make up the greater population. It is important that energies be placed to train the best and the most dedicated individuals, rather than merely looking for scientists to apply for grants in plant basic sciences. Many cancer researchers (for example) who have not been highly funded are now becoming interested in plants. Individuals interested in plants through more positive incentives will be the ones to make most of the contributions. This should not be looked upon as a means to restrict the types of talents required. Success in both the basic and applied plant sciences will depend on interdisciplinary efforts from a wide variety of fields.

Proprietary interests of industry as well as the nature of industrial research have traditionally kept much information from the public. Offsetting this situation is the fact that patented information does become available and that there is now much more industrially funded university research. Only a few years ago, materials produced through publically funded basic research generally were immediately available to other public researchers and to private industry as well. Now, many scientists realizing the potential for financial gain to themselves or their institutions distribute materials only after they have decided that there is no value or after patents have been filed and/or agreements made. This does set back research slightly; however, the results are made available after rights are formalized. This situation should be compared to a discovery made in a private company. In many cases, the outside world never knows about or obtains the material; especially material that the inventing company does not see an application for. Discoveries made in universities are almost always published. Other investigators, if they cannot obtain the material, can follow the publication to obtain their own similar material. In most industries, publications are kept to a minimum and the work is not known to the outside, unless it is patented.

The small biotechnology companies have made important contributions to basic research. Much of the interesting information on the molecular biology of interferon genes has been published by scientists working with these biotechnology companies. Again, from my own experience, scientists working in the biotechnology company that I am associated with are writing papers for public journals. We, and other biotechnology companies as well, encourage publication. The scientists frequently make scientifically important observations that have no foreseeable application and warrant publication. Without the existence of the company, those observations might never have been made. A publication record by a biotechnology company helps to attract dynamic researchers and consultants since it helps establish the company as a co-contributor to the expansion of science; individual scientists can establish their own expertise outside of the company as creative scientists. At many of the scientific meetings on biotechnology, corporate scientists (maily from small companies) are key scientific speakers.

Universities must be funded so that researchers can follow interesting observations not defined by industry. The U.S. is not the obvious world leader in basic plant sciences as it is in health sciences. Funding should be sufficient to keep up with the leading laboratories around the world. Trained dedicated individuals need to be on top of the field if we are to expect our industries to remain competitive with foreign ventures in agricultural biotechnology. Agricultural biotechnology may be the key to a new type of agriculture, and as agriculture is an industry crucial to the U.S. economy, basic plant science research must be supported by the government if we do not want to see our own agriculture decline relative to other countries.

There seem to be tremendous opportunities for universities, industries, and government laboratories work together and have the public benefit from these relationships. There are many types of interactions possible. Whatever the relationship, a public institution (or work supported by public funds) should not allow secrets to be kept from the public (with the possible exception of the short lag necessitated by patent applications) or to restrict a graduate students' ability to discuss his or her research with anyone at any time (even a company competing with a company for whom the professor is a consultant). A Faculty member's corporate affiliation should be openly known by his colleagues and students. A corporation should not be able to "buy" a department's knowledge. It is important that private industry does not dictate the research direction of universities. There obviously will be some influence, but future progress in applied research depends on unrestricted basic work. There must be a source of funding to maintain excellence in areas of basic plant sciences with no obvious immediate value to industry. This supplies human and experimental resources for the future of applied biotechnology--a future requiring skills and knowledge that no one can now predict and on which we all depend on for our well-being.

Winston J. Brill

Nitrogen Fixation: Basic to Applied

The increasing cost of nitrogen fertilizer—which is essential for high-yielding cereals—has triggered research into the mechanism of natural nitrogen fixation

All human beings require certain preformed amino acids in order to synthesize proteins. These amino acids are supplied by foods derived mainly from plant protein (for example, bread) or from animals (beef) that are fed plant protein (hay). Most plants synthesize their amino acids from compounds in the soil that contain nitrogen (N), such as ammonia (NH_3) or nitrate (NO_3^-). There are some natural sources of N compounds for plant growth. For example, plants in soils that have not been artificially fertilized obtain their N for amino acid biosynthesis from microbial degradation of dead plant or animal material. Lightning storms produce nitrates that also add, in a small way, to the fertility of the soil. Most soils, however, are deficient in these compounds, and industrially produced N fertilizers are necessary to grow high-yielding cereals (and to keep golf courses green).

Before modern Western agriculture, N for food production was obtained from recycled wastes. In the late nineteenth century, farmers used rock nitrates as fertilizer. But as the population increased and these sources became depleted, the need for new sources of N fertilizer became apparent. The element N is not in short supply, as N_2 (nitrogen gas) constitutes 80% of the air we breathe. But no animal or plant is, by itself, able to use atmospheric N_2 as an N source for protein production. This is because no higher organism has the enzymatic machinery to break the extremely strong triple bond between the two N atoms and "fix" N_2 into nitrate, ammonia, or amino acids—examples of fixed N compounds. The process of converting N_2 to fixed N compounds is known as N_2 fixation.

In the early 1900s, the German scientists Fritz Haber and Carl Bosch discovered a way to convert N_2 into ammonia by use of a metal catalyst. This discovery was soon developed on an industrial scale. The first major incentive for commercialization was the production of nitrate for weapons, and the reaction remains the main source of N fertilizer for today's intensive agriculture. The reaction requires several hundred degrees Celsius and several hundred atmospheres of pressure as well as a source of H (hydrogen) atoms—requirements that are met by the use of fossil fuels such as oil and natural gas. As long as petroleum feedstocks were plentiful and relatively cheap, fertilizer costs remained low. In the last few years, however, fossil fuel costs have risen dramatically, with greater increases predicted for the future, and we have become aware that the supply is not unlimited. So long as the production of N fertilizer depends on fossil fuels, the rising costs and limited supplies will affect the worldwide price of food.

The major source of fixed N production, however, is of biological origin. Certain bacteria contain the enzyme nitrogenase, which breaks the triple bond in atmospheric N_2 and reduces it to two molecules of ammonia. Other enzymes then convert the ammonia to amino acids. These N_2-fixing bacteria are found in a wide range of environments. Many bacteria, such as *Klebsiella pneumoniae*, can grow in soils deficient in fixed N since they can, by themselves, utilize the N_2 in the air. Some N_2-fixing bacteria, as individuals, cannot live on N_2 as a sole N source but require a specific partner to enter into a N_2-fixing symbiosis. The most important example of this is the symbiosis between the legumes and the bacterium *Rhizobium* (Fig. 1).

Legumes include such plants as soybean, alfalfa, clover, pea, bean, peanut, and lentil. When the roots of these plants are infected by the bacterium *Rhizobium*, tumorlike growths form. These growths, or root nodules, enclose the bacteria and enable the *Rhizobium* to fix N_2 to ammonia. The plant then utilizes the ammonia for protein synthesis. Because the associated bacteria fix N_2, legumes grow without the addition of N fertilizer to the soil. In fact, legumes are commonly used to fertilize a soil by crop rotation. Presumably because N (as N_2) is always available, legumes are unusually rich in protein—protein independent of industrial chemical N_2 fixation.

Often, farmers apply more industrially produced fertilizer to fields than the growing plants can use. This is supposed to offset loss of N by runoff (which creates a pollution problem) and leaching after rains, and loss by denitrification, a micro-

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ase, which oxidizes H_2 and in the process yields either H^+ ions and electrons capable of reducing nitrogenase or energy in the form of ATP to drive N_2 fixation. Certain strains of *Rhizobium* that have such hydrogenases may fix more N_2 for the legumes than strains that lack hydrogenase (Schubert and Evans 1976). Apparently hydrogenase-containing strains counteract the waste of ATP and electron-dependent H_2 evolution. This feature may be a factor in choosing *Rhizobium* strains to be produced commercially for agricultural use.

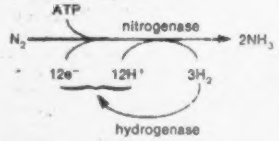


Figure 2. Some of the electrons (e^-) and H ions (H^+) that reach nitrogenase are not used for ammonia production; rather, they seem to be wasted by combining to form H_2 gas. Bacteria that have the enzyme hydrogenase are able to recycle H_2 for further use by nitrogenase.

Some of the first questions asked by biochemists about a metabolic reaction concern the nature of the intermediate products. The steps and intermediates in the reduction of N_2 to ammonia are still unknown. It appears that all of the intermediates are enzyme-bound, and not free in the cytoplasm. Recently, however, Thorneley and co-workers (1978) demonstrated the presence of an intermediate by hydrolyzing N_2-fixing nitrogenase to yield hydrazine (N_2H_4), which indicates that a N_2-hydride intermediate might be involved. Much more work is needed before the sequence of reactions converting N_2 to ammonia is understood.

N_2 is not the only substrate that can be reduced by nitrogenase. The enzyme also reduces many nonphysiological substrates, most of which have a triple bond between C and/or N atoms. In all cases, ATP, a source of H atoms, and components I and II are required. Nitrogenase can reduce cyanide to methane and ammonia (Hardy and Knight 1967), azide to ammonia (Schöllhorn and Burris 1967), and acetylene to ethylene (Dilworth 1966).

Acetylene reduction is valuable as an index of N_2 fixation. Acetylene is a water-soluble gas, whereas ethylene is not very soluble, and these properties lend themselves to a commonly used assay for N_2-fixing activity. A sample of soil, a plant root system, a pure culture of bacteria, or pure nitrogenase can be placed in a closed container and acetylene is added. After an incubation period, a sample of gas is removed and injected into a gas chromatograph. Within minutes it is possible to determine, with great sensitivity, the amount of ethylene produced: the more ethylene, the greater the enzymatic activity. This acetylene-reduction assay has been useful for studying the mechanism of nitrogenase action as well as for examining N_2 fixation in situ under field conditions. The assay is simple and inexpensive and therefore especially useful to investigators in developing countries.

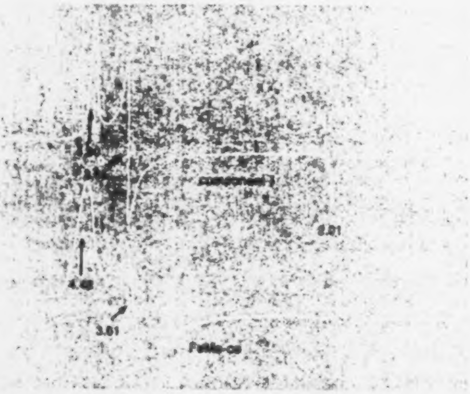


Figure 3. Comparison of the electron paramagnetic resonance spectra of component I and the iron-molybdenum cofactor (FeMo-co) shows striking similarities, an indication that the transition metals producing this spectrum remain in the same sort of structure in FeMo-co as they are in the native enzyme. Studies with mutants have shown that this structure is an active site of nitrogenase. The numbers on the figure are spectroscopic splitting values.

Electron paramagnetic resonance (EPR) spectroscopy is another useful tool for studies of nitrogenase. EPR spectroscopy (Orme-Johnson et al. 1972), along with experiments on enzyme kinetics (Hageman and Burris 1978), indicates that the role of component II is to transfer electrons to component I. N_2 fixation actually occurs in component I. EPR spectra of component I have a unique signal with a spectroscopic splitting value of 3.65. Studies with mutants indicate that this signal is caused by the active site of nitrogenase (Shah et al. 1973)—the active site of an enzyme is where the reaction takes place. We shall come back to a further discussion of this signal.

Molybdenum is an unusual metal to be found in a protein, and scientists therefore suspected it to be part of the active site of nitrogenase. Other work indicated that molybdenum-containing enzymes have their molybdenum in some type of small cofactor (Nason et al. 1971). The question was whether nitrogenase also contains molybdenum in a cofactor, and to answer it, Nagatani and colleagues (1974) found a mutant of a N_2-fixing bacterium that was unable to fix N_2. The nitrogenase activity in an extract of the mutant could be reconstituted upon addition of normal component I that had been denatured by acid. Acid apparently released a cofactor from component I that was missing in the extract of the mutant. The cofactor, released in this manner, was very unstable.

A method has now been worked out for isolating the cofactor and retaining its activity in a stable form (Shah and Brill 1977). The cofactor, known as FeMo-co, contains iron, molybdenum, and sulfide in the ratio of 8:1:6. Its molecular weight has not yet been directly determined, but it seems to be less than 5,000. The novel technique of EXAFS (extended x-ray absorption fine structure), performed with the Stanford Linear Accelerator, indicates that the molybdenum is bound to sulfur atoms (Cramer et al. 1978).

FeMo-co in the presence of an H donor catalyzes acetylene reduction to ethylene (Shah et al. 1978), and the process requires neither ATP nor component II. This experiment suggests that FeMo-co is an active site. Because analysis of the EPR spectrum of FeMo-co revealed a signal very similar to the 3.65 signal, it can be said that FeMo-co contains the active site of nitrogenase in a form similar to that found in the native enzyme (Fig. 3). Mössbauer spectroscopy showed that iron atoms were responsible for the EPR signal (Rawlings et al. 1978). Thorough characterization of FeMo-co will simplify studies of nitrogenase activity and may indicate ways to produce more efficient catalysts for use in industrial N_2 fixation.

Genetics and regulation

Mutants of N_2-fixing bacteria have already yielded a wealth of information about the biochemistry and physiology of N_2 fixation. Work with mutants may also suggest ways of genetically improving N_2-fixing organisms for further benefit. *Klebsiella pneumoniae*, which is closely related to the well-studied intestinal bacterium *Escherichia coli*, has been the most extensively studied bacterium with respect to the genetics of N_2 fixation. Streicher and co-workers (1971) showed that mutations, specifically preventing the ability of *K. pneumoniae* to grow on N_2, cluster in a small region of the bacterial chromosome. Such mutations are called *nif* (*ni*trogen *f*ixation) mutations.

Cannon and colleagues (1974) used genetic techniques to put the DNA containing the *nif* region onto a plasmid, a small piece of DNA that is normally not a part of the main chromosome. When this *nif*-containing plasmid was introduced into *E. coli*, which normally is unable to fix N_2 because it has no *nif* genes, the resulting *E. coli* could fix N_2 when the cells were grown in the absence of O_2.

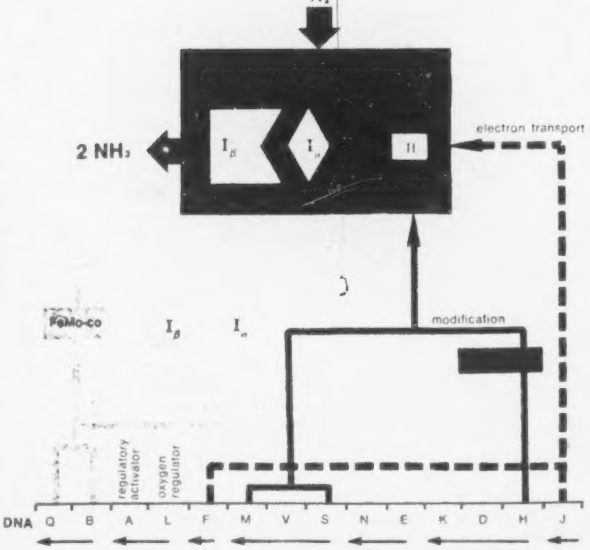


Figure 4. The 14 *nif* genes of *Klebsiella pneumoniae* are in a cluster that is less than 1% of the total cell chromosome. The arrows along the bottom represent the 7 individual operons, with the direction of messenger RNA and protein synthesis indicated. The protein structure of component II is coded for by *nifH*, but the products of the *nifM* and *nifS* genes are necessary to modify and activate component II. The structure of the two proteins in component I is specified by *nifK* and *nifD*. In order to obtain active component I, FeMo-co is required. FeMo-co synthesis demands the products of the *nifQ*, *nifB*, *nifN*, and *nifE* genes. Proteins coded by the *nifF* and *nifJ* genes are necessary to transport electrons to nitrogenase. The electrons break the triple bond between the two N atoms, converting each atom to two molecules of ammonia. The activator produced by *nifA* turns on the production of the *nif*-coded proteins when the cells lack sufficient fixed N in their environment. The function of the *nifL* product is to prevent nitrogenase synthesis when the cells become aerobic. The function of *nifV* is not known at present.

This experiment has tremendous potential significance. Will it be possible to transfer the *nif* genes to plants such as corn or wheat and enable them to fix N_2, thereby becoming independent of N fertilizer?

The rapid advances in genetic engineering will probably soon make possible the transfer of *nif* genes into cultured plant cells in the laboratory. The technology for propagating certain plant cells into mature plants already exists. Thus, a genetically engineered cereal plant that can fix N_2 seems, theoretically, imminent. But a plant that might be expected to be useful in field conditions probably remains in the distant future. Why do no existing cereal plants fix N_2? What barriers have selected against this reaction in higher plants? The properties of nitrogenase suggest many problems that must be overcome. First, some mechanism must keep nitrogenase from being inactivated by the O_2 produced by photosynthesis. Also, because nitrogenase is a rather sluggish enzyme, it generally comprises a relatively large percentage of the total protein of cells that grow on N_2. Thus, a genetically engineered N_2-fixing corn plant may have to take up much more iron and molybdenum than usual. It will also have to satisfy the high requirement for ATP. Furthermore, once N_2 fixation is achieved in cultured plant cells, the cells must still be able to develop properly if the plant is to be agronomically useful.

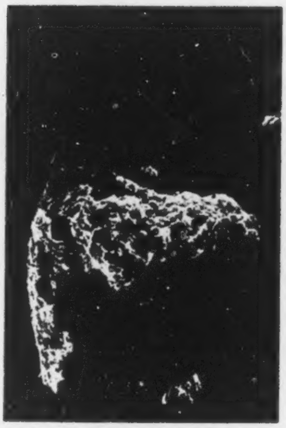


Figure 5. Scanning electron micrographs demonstrate the structure of a nodule on an alfalfa root. *From left to right:* The whole nodule shown is about 22 mm across. Cut in half, it reveals the enlarged plant cells with which it is filled. Within each of these cells are many *Rhizobium* cells, which contain nitrogenase and fix nitrogen for the legume. At the end of the sequence, a photo made by transmission electron microscopy of a sectioned *Rhizobium* cell shows the material surrounding the cell which contains the polysaccharide that binds the bacterium to the plant root. The length of a *Rhizobium* cell is approximately 1 μm. (Photomicrographs by F. B. Dazzo and E. Hrabak.)

Nitrogenase component I actually is made up of two different polypeptides. These polypeptides, plus the one polypeptide in component II, dictate that at least three genes are required for the synthesis of nitrogenase. A detailed genetic analysis of the *nif* region in *K. pneumoniae* demonstrated that at least 14 *nif* genes, all clustered closely together, are specifically required for this organism to grow on N_2 (MacNeil et al. 1978) (see Fig. 4). This is a surprising result; it points to the great complexity of biological N_2 fixation that must be dealt with in the engineering of a *nif*-containing cereal plant.

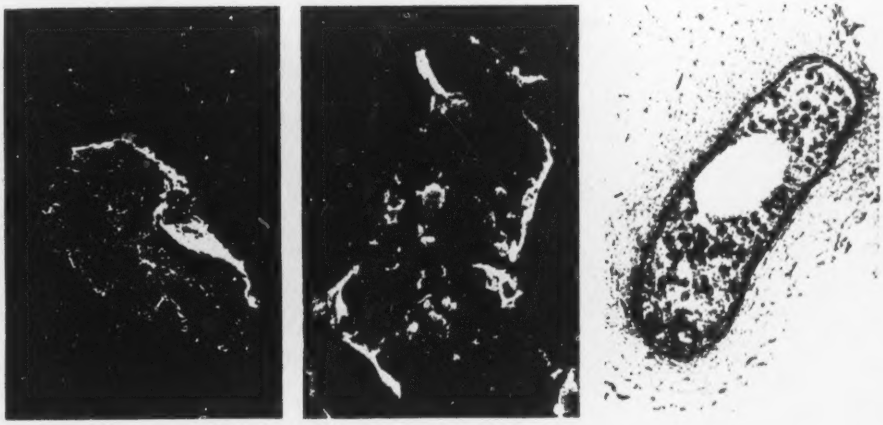
Four of the 14 genes are required for the production of FeMo-co (Roberts et al. 1978), which is a part of the active site. Other genes seem to be involved in processing nitrogenase components. Two genes are needed for the cells to grow on N_2, but they are not required for N_2 fixation in cell extracts. The nitrogenase assay in cell extracts bypasses the natural electron donors: it is likely that these gene products are involved with electron transport or ATP production for nitrogenase activity in the living cell. Analyses of hundreds of mutants made it obvious that the *nif* genes code for more than one messenger RNA (mRNA) (MacNeil et al. 1978). Seven different operons (yielding seven different mRNAs) make up the *nif* genes. Each operon is read in the same direction.

N_2 fixation is an energy-taxing reaction for a cell. It makes sense, then, that bacteria fix N_2 only when they need to. Under some conditions, N_2 fixation does a cell no good. If, for instance, there is sufficient ammonia in its environment, *K. pneumoniae* does not synthesize nitrogenase (Pengra and Wilson 1958) but utilizes the fixed N already available. When the bacterium uses up the fixed N, it begins to synthesize nitrogenase and utilize N_2. Blue-green algae react in a similar way: they do not form heterocysts in environments containing fixed N sufficient for growth. Legumes do not form nodules when fields are treated with high levels of N fertilizer.

We do not yet understand the mechanism by which the bacterium switches on and off the synthesis of nitrogenase, but the enzyme glutamine synthetase apparently plays a role in this regulation (Tubb 1974). Glutamine synthetase is ultimately responsible for converting the ammonia formed from N_2 fixation to amino acids for protein synthesis. In addition to this catalytic activity, the enzyme is also presumed to interact with the *nif* DNA. We know that the protein product of the *nifA* gene is involved in regulation because mutations in this gene completely eliminate the production of the other *nif*-coded proteins (Roberts et al. 1978). Several laboratories are studying the nature of interactions between glutamine synthetase and the *nifA* gene. The *nifL* gene also plays a role in regulation. As previously mentioned, nitrogenase is readily inactivated by O_2. The *nifL* gene produces a protein that prevents nitrogenase synthesis in the presence of air (Roberts, unpubl.) and thus stops the bacterium from wasting energy by synthesizing nitrogenase that would be rapidly inactivated.

Regulation of nitrogenase synthesis, which is very tightly controlled, prevents a N_2-fixing bacterium from producing more ammonia than actually is required for growth. It is, however, possible to obtain mutants that overcome the inhibition of nitrogenase synthesis by fixed N. One type of mutant has a defect in glutamine synthetase and thus requires an amino acid in order to grow (Shanmugam and Valentine 1975). Another type, in the *nifA-nifL* region of the DNA (MacNeil, unpubl.), has no special growth requirement. Mutants with such defects synthesize nitrogenase whether or not high concentrations of fixed N are present in their environment.

Mutations like those with uncontrolled nitrogenase synthesis can perhaps be used in new applications of N_2-fixing bacteria. For example, if such strains excrete ammonia, they might be useful for increasing the N content of a carbon-rich waste product, such as paper-mill waste, which



would allow for more rapid degradation of the waste. Alternatively, the N-enriched waste could be used as fertilizer. An ammonia-excreting bacterium might be made to grow on organic compounds from cereal plant roots and supply fixed N to the plant. The *Rhizobium*-legume symbiosis, unregulated by fixed N, could add N to a fertilized soil rather than using up the fixed N already in the soil. These are just a few potential applications that may be possible with mutant organisms which have defects in the control of their *nif* genes.

Bacteria–plant symbioses

Certain bacteria contain the *nif* genes but are unable to grow on N_2 by themselves. In order for such bacteria to benefit from N_2 fixation, they must enter into a symbiosis with a higher organism. The best known example is the *Rhizobium*-legume symbiosis, in which photosynthate (carbon compounds formed from plant photosynthesis) reaches the nodule and supplies the energy and electrons for N_2 fixation by the bacteria. The plant nodule produces a microenvironment that protects *Rhizobium* by keeping other bacteria from getting into the nodule and competing with the *Rhizobium*. In return, the plant obtains ammonia that is used for amino acid synthesis for plant protein production.

The *Rhizobium*-legume symbiosis exhibits specificity. For instance, the *Rhizobium* that nodulates clover does not nodulate soybean. Farmers and gardeners buy commercially prepared specific *Rhizobium* inoculants to guarantee that their legumes will fix N_2. Bohlool and Schmidt (1974) showed that a protein from soybean seeds binds to *Rhizobium* strains that nodulate soybean but not to strains of *Rhizobium* that nodulate other legumes. The root surface of legumes apparently contains proteins capable of recognizing a polysaccharide on the surface of a specific *Rhizobium* strain (Dazzo and Brill 1977). It is interesting to note that this polysaccharide is very similar to one (the O-antigen) that binds certain pathogenic bacteria to specific mammalian host receptors (Wolpert and Albersheim 1976; Maier and Brill 1978a).

Very little is known about the details of the steps that actually lead to a N_2-fixing nodule. After the *Rhizobium* attaches to a legume root (via a protein), the plant cell wall invaginates to produce a tube within the cell (Fred et al. 1932). This tube, which is called an infection thread, has a wall of cellulose. Many infection threads abort, but some begin spilling the *Rhizobium* into plant cells and infect neighboring cells. The bacteria multiply and completely fill these plant cells, which then increase in size (see Fig. 5). The bacteria produce nitrogenase, which provides N for the plant—while the plant synthesizes leghemoglobin to protect nitrogenase from O_2.

Many researchers are trying to improve the N_2-fixing symbiosis by such approaches as plant breeding to supply, for instance, more photosynthate to the nodule. Bacterial inoculants and inoculation practices also are being improved. Maier and Brill (1978b) obtained mutants of *Rhizobium* that increased N content in soybeans by about 100% over that in soybeans inoculated with the parent strain of the mutants (Fig. 6). These experiments have been carried out only with potted plants in growth chambers, but experiments are being performed at present to determine whether the superior characteristics of such mutants can be translated into greater protein yields in plants grown in field conditions. One major problem has yet to be overcome: the experimental strains must compete successfully against strains of *Rhizobium* that are already indigenous to field soils. Our understanding of factors that influence competition between bacterial strains in the soil is quite sparse and still requires major research efforts.

Legumes are not the only plants that enter into a nodule-forming, N_2-fixing symbiosis with bacteria. The alder tree (*Alnus*), which already plays an important role in the lumber industry in the northwest United States, may have even greater and more versatile use because N_2-fixing nodules make it independent of fertilizer N addition (Figs. 7 and 8). The nodules differ from legume nodules in several ways. No leghemoglobin is present, and it is not understood how the alder protects nitrogenase from O_2. The bacteria in these nodules have recently been isolated (Callaham et al. 1978); they are not *Rhizobium* and they do not nodulate legumes.

Over the past few years considerable activity has been devoted to finding new N_2-fixing symbioses for agricultural use. Of the many thousands of different kinds of legumes, only a small percentage have ever been tested for agronomic potential. In a survey of underexploited tropical plants, the U. S. National Academy of Sciences (1975) noticed the tremendous potential of a legume called the winged bean (*Psophocarpus tetragonolobus*). This plant may be very useful for food production in the tropics since it grows well without N fertilizer. The pods, leaves, and seeds of the winged bean are edible and are very rich in protein. The seeds are also rich in edible oil, and the tuberous roots yield about 20% (dry weight) protein. Further surveys promise to discover more plants of special interest—perhaps plants that can thrive under such normally adverse conditions as dry, saline, or acid soils.

Another N_2-fixing symbiosis, the *Azolla–Anabaena* system (see cover), has received attention in the last few years. *Azolla* is a small floating water fern that grows in many parts of the world. Because small pores in its fronds contain the N_2-fixing blue-green algae *Anabaena*, the fern thrives in waters deficient in fixed N. *Azolla* has been used for centuries in rice culture in China and Vietnam. Before planting rice, the farmer allows the surface of the rice paddy to become covered with the fern; the mature *Azolla* cover is approximately 2 cm thick and contains about 50 kg N per hectare. As the rice plants grow, they eventually shade the *Azolla*, and the fern dies, releasing into the paddy fixed N, which is then used by the rice plants. This practice permits rice to be grown season after season in the same paddy without requiring the addition of fertilizer. Another way to exploit this symbiotic fern-algae is to harvest the fern plants, by skimming the surface of a pond, and to use them as a green manure.

Associations of mutual benefit

Some relationships between N_2-fixing bacteria and plants are less intimate than the symbioses discussed above. In these looser relationships no specialized root structures protect the bacteria from competition with other soil bacteria, and there is no direct channeling of photosynthate from the leaves to the bacteria or of ammonia from the bacteria to the plant. Such systems are termed N_2-fixing associations.

Certain grasses—sugar cane, for example (Döbereiner et al. 1972)—have N_2-fixing bacteria growing around their roots, and the bacteria seem to play a role in fertilizing the plants. Presumably, the plants excrete organic photosynthate and thereby supply energy and electrons for N_2 fixation. Researchers are attempting to determine whether such associations can be established for corn (Von Bülow and Döbereiner 1975) and other important cereal crops (Smith et al. 1976). At present, the value of inoculating these crops with N_2-fixing bacteria is questionable (Barber et al. 1976; Albrecht et al. 1977), but it may be possible to obtain corn or wheat plants with smaller demands for N fertilizer by developing an association through isolation (and perhaps genetic modification) of the proper N_2-fixing bacteria.

Perhaps the most important factor in establishing these beneficial associations is the use of plants specifically developed for them. For instance, today's high-yielding varieties of cereal plants probably excrete only minimal amounts of photosynthate through their roots, but it may be possible to select plants that excrete larger amounts of photosynthate that can be used by the N_2-fixing bacteria. Such plants may sacrifice seed yield,

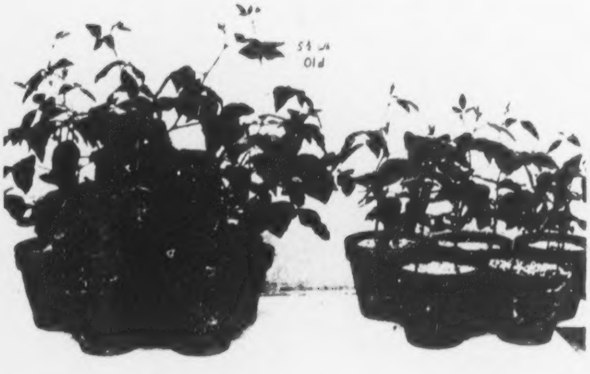


Figure 6. Fixation of N_2 in soybean can be improved by isolating mutants of *Rhizobium* that increase the N content. The soybean plants on the right were inoculated with a strain that has been used for a number of years in agriculture; those on the left were inoculated with a mutant derived from this strain. The mutant-inoculated plants have about twice as much fixed N as the plants inoculated with the wild type. For such mutants to be effective in agriculture, however, they must be able to compete successfully with other bacteria under field conditions. (Photo by the author.)

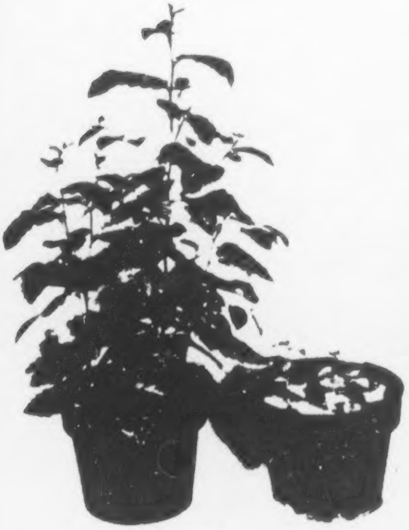


Figure 7. The alder (*Alnus oregona*) on the right was supplied with a nutrient solution containing N but was not inoculated with N_2-fixing bacteria. The plant on the left was supplied with the same solution but was inoculated with a crushed nodule suspension. These plants are about 4 months old.

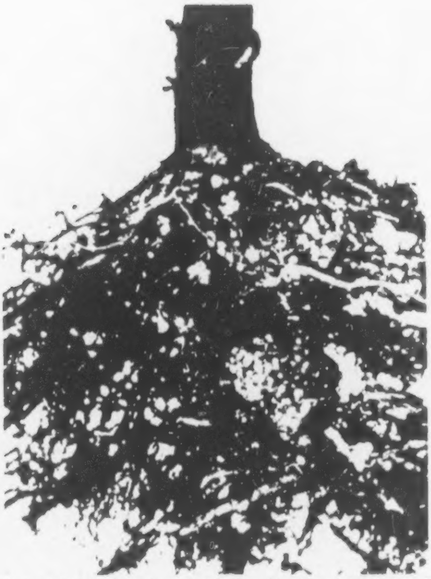


Figure 8. The N_2-fixing bacterium harbored in the nodulated roots of the alder is not the same kind as that found in the roots of legumes, *Rhizobium*. Leghemoglobin is not found in alder nodules, and we do not know how the nitrogenase is protected from O_2. (This photo and the one in Fig. 7 were taken by S. Russell and H. J. Evans.)

but with fertilizer prices rapidly increasing this may someday be an economically attractive practice.

The threat of increased protein shortages is a worldwide concern that has recently stimulated many governments to support research on protein production by biological N_2 fixation with the hope that protein shortages can be alleviated. As scientists understand more about the basic processes of N_2 fixation in different systems, they generate and rapidly test new ideas for application. The investigation of N_2 fixation is truly an international effort, with researchers from many nations (developing and developed) collaborating on projects. Collaboration is found in another form as well: integrated efforts among chemists, agronomists, bacteriologists, geneticists, soil scientists, biochemists, and other scientists are steadily yielding new discoveries and outlooks. Continued imaginative research on this fundamental biological reaction is bound to yield applications for increasing food production.

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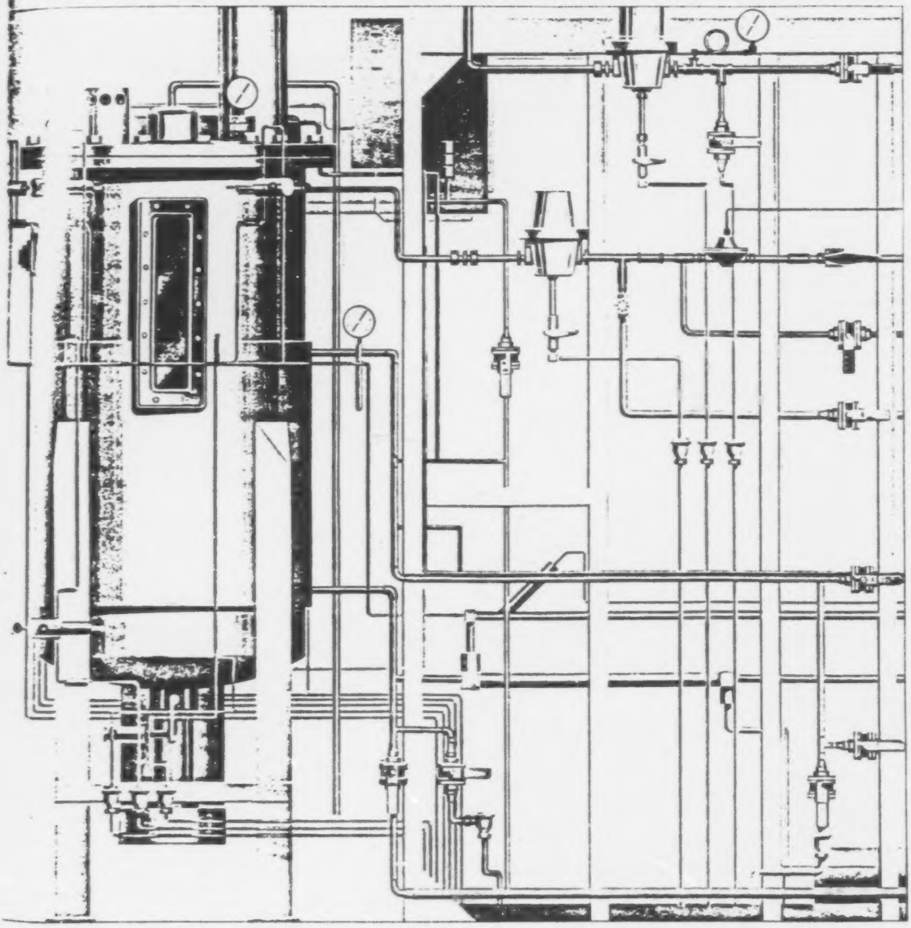
"And, to round out the four basic food groups, I recommend the gnocchi de semoule avec pâte à chou-Patalina."

SCIENTIFIC AMERICAN



INDUSTRIAL MICROBIOLOGY

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September 1981

Agricultural Microbiology

Introducing new genes into crop plants by recombinant-DNA methods is difficult and not in immediate prospect. Much progress can be made, however, by manipulating the microorganisms that live with plants

by Winston J. Brill

A handful of soil scooped from the fields of a mechanized farm is the locus of an unruly turmoil of competing microorganisms. In the soil thousands of strains contend for nutrients and energy, in the process altering the chemistry of the soil with the products of their metabolism. Moreover, the microorganisms themselves evolve in response to stresses imposed by their environment, including the stresses induced by the evolution of their fellow species. Suppose into the teeming genetic marketplace a new colony of bacteria is introduced, one selected, say, for the ability to invade the roots of certain crop plants. Even if the new bacteria can survive the competition and adapt to the changeable environment, they may not be able to carry out their intended function. They may find that the root system is already occupied by other strains of microorganisms.

Interference of this kind is commonplace in agricultural practice; it serves to illustrate the difference between the open field and the fermentation tank. There are also similarities: in both agriculture and industrial microbiology the aim is to meet human needs by the selective breeding, culturing and harvesting of living organisms. If the fermentation tank can be taken to represent an agricultural environment, however, it is one subject to exceptionally precise control. For example, the population of microorganisms is usually limited to a single species. In considering the potential applications of microbiology to agriculture, one must begin to explore the subtle question of the interaction of microorganisms with one another and with the biosphere as a whole.

The growing demand for food and other agricultural products is ample practical justification for undertaking the enormous research effort that will be needed in order to apply the methods of microbiology to agriculture. It has been suggested that the "engineering" of crop plants and of the soil microorganisms on which they depend may yield hybrid grains capable of obtaining their supply of nitrogen directly from the atmosphere. No crop plant now has this capacity; the nitrogen must be fixed, or converted into a biologically useful form, either by microorganisms or by the industrial manufacture of nitrogenous fertilizers, a process that calls for a large expenditure of fossil fuel. American farmers spend about $1 billion a year on nitrogenous fertilizers for the corn crop alone, and so the program of research on nitrogen fixation is a growing one.

Other lines of biological investigation may lead to the acceleration of photosynthesis and to the development of crops that can be grown on saline or highly acidic soil. These are ambitious goals, unlikely to be achieved commercially in less than 10 years. Nevertheless, a great deal of rational exploitation and modification of the microbiological environment of the farm is within reach during the next decade. The agricultural community has only recently begun to recognize the potential of microbiological techniques in plant-cell research. Recombinant-DNA technology may lead to improvements in existing crops and to the development of entirely new crop types. So far, however, its most important effect has probably been on industrial laboratories, which have been alerted to the possibility of applying biological methods to agriculture. Work of this kind is now under way in several dozen such establishments, focusing primarily on the design of microorganisms important to agriculture or on the application of microbiological techniques to the manipulation of plants. Perhaps the most significant indicator of the revolutionary nature of the work is that almost all these laboratories have been established within the past two years.

How can microbiological techniques be applied to traditional agricultural practices? There are three main strategies. First, microorganisms found to be beneficial to plants (or designed for this purpose) can be bred and grown in fermentation tanks for later introduction into the soil. Second, individual cells can be isolated from plant tissue and grown in nutrient solution. In such cell cultures the rate of mutation can be increased, making possible the selection of promising strains, the development of hybrid strains that would not be obtainable by standard breeding techniques and the manufacture in large fermenters of certain plant products, such as digitalis, pyrethrins (which are natural pesticides) and licorice.

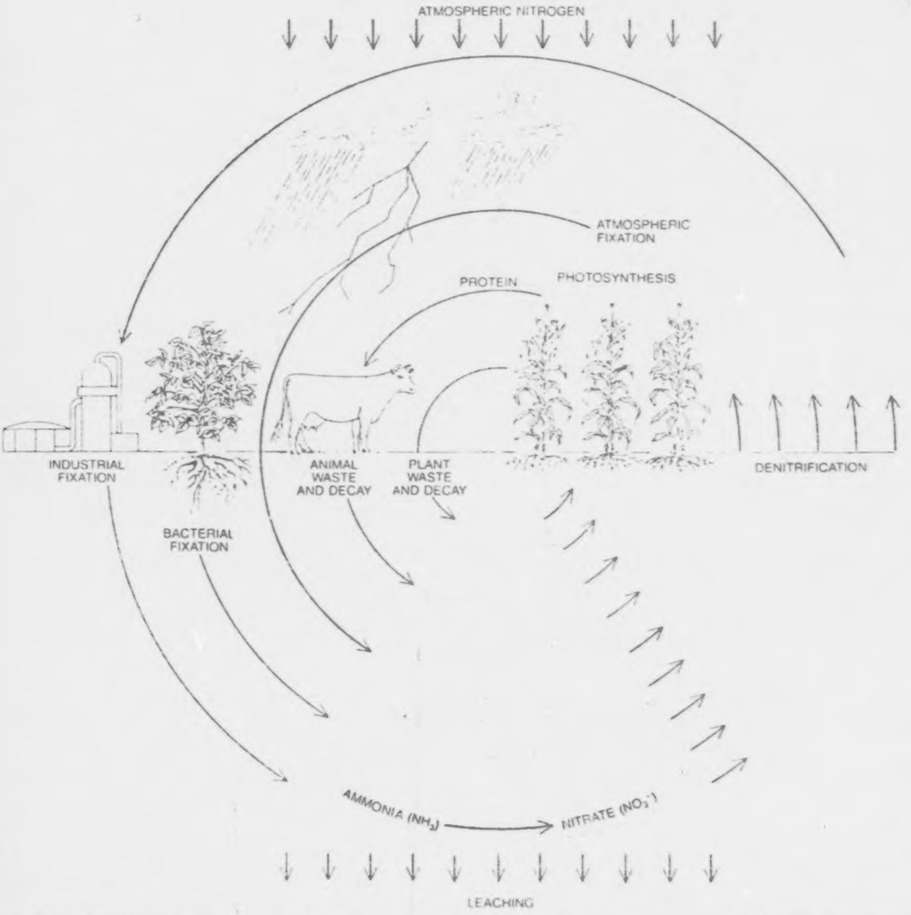
Third, foreign genetic material can in some cases be introduced into plant cells, a practice that could open the way for direct genetic engineering of the plants themselves. Such techniques are still primitive when they are compared with the achievements of recombinant-DNA technology with bacteria. Indeed, the reverse strategy of inserting plant genes into bacteria, so that plant proteins can be made by culturing bacteria in standard fermentation tanks, may

MICROORGANISMS ESSENTIAL TO AGRICULTURE include bacteria of the genus *Rhizobium*, which are seen infecting a root hair of a clover plant. In the photomicrograph, which was made by B. Ben Bohlool of the University of Hawaii at Manoa, the bacteria have been labeled with a fluorescent dye; they appear as bright chartreuse flecks. They form an infection thread that extends from the tip of the root hair into the interior of the root. *Rhizobium* bacteria live symbiotically in the roots of clover and other legumes; the bacteria supply the plant with fixed nitrogen necessary to photosynthesis and the plant nourishes the bacteria. Such interactions of microorganisms with plants suggest several ways the new methods of genetic engineering might contribute to agricultural practice. For example, the nitrogen-fixing capacity of the bacteria might be increased by genetic modification, or the bacteria might be induced to colonize plants other than legumes. If the modified organisms are to be effective, however, they must be able to compete successfully with strains indigenous to the open field.

well precede the genetic engineering of plant DNA.

The exploitation of microorganisms in the soil is hardly new to agriculture. It was well known in Roman times that legumes such as beans, peanuts, alfalfa, soybeans, peas, clover and lupine enhanced the fertility of the soil. Soil from fields where legumes had been grown was added to fields that were to be assigned to legume cultivation for the first time. The Romans could not have known that the underlying justification of their sound empirical practice is the presence of bacteria of the genus *Rhizobium*, which infect the roots of some legumes and fix atmospheric nitrogen [see "Biological Nitrogen Fixation," by Winston J. Brill; SCIENTIFIC AMERICAN, March, 1977]. As the term infect suggests, the introduction of *Rhizobium* into legumes resembles a disease process, but it is one in which the plant cooperates. The *Rhizobium* bacteria enter into a symbiotic relation with the plant: the bacteria obtain nourishment from the plant and in turn provide the plant with usable nitrogen in the form of ammonia (NH_3). The relation is an intimate one, in which the bacteria actually enter the roots of the legume and form the visible growths called nodules.

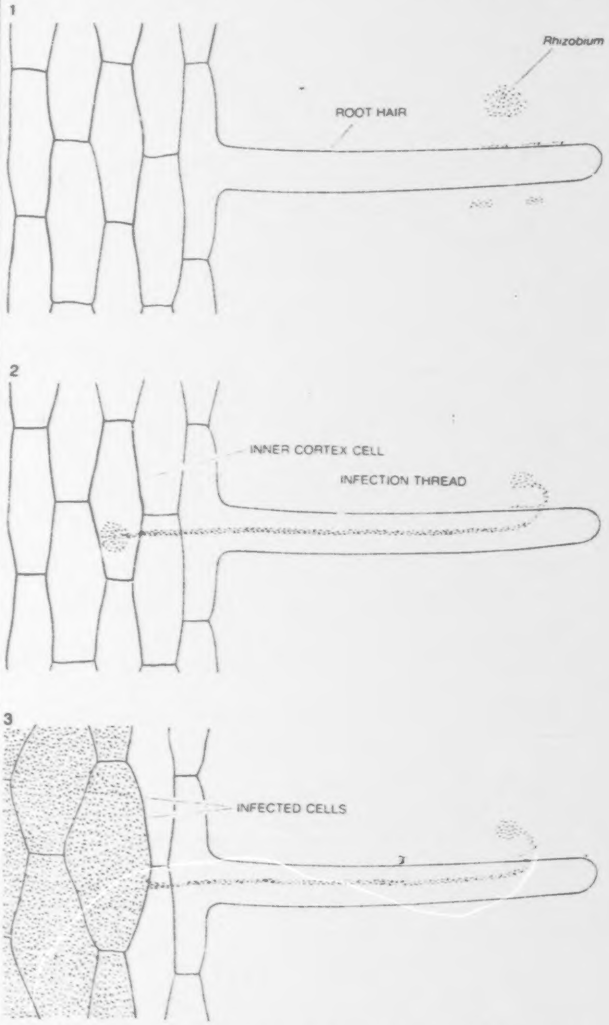
For the Romans a population of *Rhizobium* bacteria in the soil ensured root nodulation and subsequent nitrogen fixation in new fields of legumes. It is also



NITROGEN CYCLE maintains a balance between two vast reservoirs of nitrogen compounds: the earth's atmosphere and the earth's crust. Because green plants can utilize nitrogen only when it has been incorporated into chemical compounds such as ammonia (NH_3) the plants cannot extract nitrogen directly from the atmosphere, where it takes the form of diatomic molecules (N_2). Atmospheric nitrogen must therefore be fixed either industrially or by bacteriological or other natural processes such as lightning. Although only a small fraction of the total nitrogen supply is required by plants, fixation must be carried out constantly. Fixed nitrogen is lost through the leaching of the soil, through the harvesting of crops and through the action of denitrifying bacteria that convert fixed nitrogen into its diatomic form.

the basis of the traditional practice of crop rotation, because fixed nitrogen left in the soil by a crop of legumes can be taken up by grain plants, which do not form nodules. In 1888 ***Rhizobium*** was isolated by the German investigators Hermann Hellriegel and H. Wilfarth, and within 15 years deliberate inoculation of soil with cultured bacteria became agricultural practice. Today strains of ***Rhizobium*** are packaged with a supporting agent such as peat.

The importance of nitrogen to plant metabolism is now well understood bio-



INFECTION OF A ROOT HAIR of a legume by *Rhizobium* begins when the bacteria attach themselves to the root hair (*1*) by means of a template-matching mechanism. The plant and the bacteria recognize each other through specific proteins. The bacteria then enter the root hair through an infection thread and stream into an internal cell of the root (*2*). The infection causes the cell to swell and divide (*3*). The result is a root nodule: a thick mass of infected cells.

chemically. Nitrogen is incorporated into a great variety of biological molecules, and it is an essential constituent of proteins, where the peptide bond that links one amino acid to the next is formed between a nitrogen atom and a carbon atom. If the amino acids of dead plants were simply returned to the soil, new crops could recycle the dead matter immediately into new proteins. In the soil, however, amino acids are dismantled to yield ammonia or nitrate ions (NO_3^-). The nitrates are further broken down by the bacteria called denitrifiers into molecular nitrogen (N_2), which is returned to the atmosphere, completing a nitrogen cycle. Moreover, when crops are harvested or when rainwater carries dissolved nitrogen compounds to deeper levels of the ground, the nitrogen-bearing matter is physically removed from the topsoil. Leaching, harvesting and the activities of denitrifying bacteria result in a net loss of fixed nitrogen, which must be replaced if the next crop is to synthesize more protein for growth.

How can the nitrogen-fixing action of *Rhizobium* on legume roots be enhanced, thereby increasing crop yield? A straightforward approach is through plant breeding, which does not require microbiological techniques at all. For example, if the rate of photosynthesis were increased by selective breeding, the bacteria in the nodules might be able to fix more nitrogen. By combining plant breeding with microbiological techniques for modifying the *Rhizobium*, however, even higher yields of plant protein might be obtained.

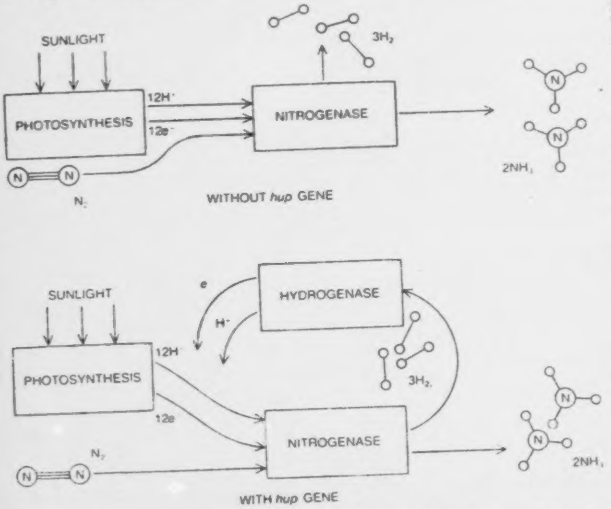
A few years ago my colleagues and I at the University of Wisconsin at Madison began to apply the screening procedures of the pharmaceutical industry to nitrogen-fixing bacteria. The bacteria were first exposed to mutagenic substances or to ionizing radiation as a means of increasing the rate of mutation in the colony. Plants were then inoculated with mutant bacteria and the amount of nitrogen fixed by each strain of bacterium was measured. In this manner we obtained several mutants capable of significantly higher levels of nitrogen fixation than those afforded by standard inoculants. The soybean plants in the experiment showed superior growth.

The experiment was done in a laboratory growth chamber, and it was important to test the effects of the mutant bacteria on plant growth under field conditions. Soybeans were planted in nitrogen-poor Wisconsin fields; one plot in each field was inoculated with the mutant bacteria and a second was not inoculated. There was no difference in growth or yield between the two plots: even the uninoculated control plot produced a good soybean crop.

We had encountered precisely the effect that distinguishes agricultural from industrial practice: the effect of natural populations of microorganisms in the uncontrolled environment of the open field. Indigenous strains of *Rhizobium* formed most of the root nodules in both test plots: the superior nitrogen-fixing mutants were unable to compete with the indigenous strains. When the mutant bacteria were introduced into fields where legumes had never been cultivated, they did lead to greater yields of soybeans. Our current plan to overcome the difficulty borrows a traditional tactic of the plant breeder: we seek to identify the most competitive strains of bacteria and to obtain mutants with better nitrogen-fixing properties from those strains.

The procedure of inducing random mutations and then screening for useful mutant bacteria is inefficient. Microbiologists in a number of laboratories are studying the genetics and the biochemistry of infection by *Rhizobium* so that the bacteria can be directly modified by genetic engineering. In all nitrogen-fixing organisms the agent responsible for fixation is the enzyme nitrogenase, which catalyzes the conversion of molecular nitrogen into ammonia. In the reaction a transport protein donates electrons to the nitrogenase, which in turn transfers them to the diatomic molecule of nitrogen by a mechanism that is not yet fully understood. Three negatively charged electrons come to be associated with each atom of nitrogen; thereafter three protons (hydrogen nuclei) are withdrawn from the intracellular medium to neutralize the charge. Hence each diatomic molecule of nitrogen yields two molecules of ammonia.

The transfer of electrons from nitrogenase to molecular nitrogen has an energetically wasteful side reaction. Many of the electrons recombine with protons before they reach the nitrogen; the recombined electrons and protons are released as molecular hydrogen gas (H_2). Certain strains of *Rhizobium* synthesize hydrogenase, an enzyme that converts molecular hydrogen back into electrons and protons for reuse by nitrogenase. Hydrogenase could therefore serve as a kind of afterburner that would make the nitrogen-fixing bacteria more energy-efficient. The enhanced bacterial efficiency would enable the plant to direct its energy more to seed yield than to the support of its symbiotic bacteria. Investigators at Oregon State University have recently demonstrated the effectiveness



WITHOUT *hup* GENE

WITH *hup* GENE

BIOCHEMICAL SHORT CIRCUIT leads to a wasteful release of hydrogen gas (H_2) as a by-product of the enzymatic reaction by means of which *Rhizobium* bacteria convert molecular nitrogen (N_2) into ammonia (NH_3). The hydrogen has no known value to the plant or to the bacteria; instead it seems merely to waste energy derived from photosynthesis. The energy serves to segregate free protons (H^+) and electrons (e^-), which then drive the reactions of nitrogen fixation. If the protons and electrons recombine to form hydrogen, however, the energy is dissipated. In some strains of *Rhizobium* bacteria a gene carried by a plasmid circumvents this inefficiency. The gene, designated *hup*, codes for the synthesis by the bacteria of the enzyme hydrogenase. Hydrogenase catalyzes the breakdown of hydrogen gas into its constituent protons and electrons for reapplication in nitrogen fixation. The introduction of the *hup* gene into a *Rhizobium* inoculant has been shown to give the inoculated plant higher seed yields than those of plants infected with strains of *Rhizobium* bacteria that do not synthesize hydrogenase.

of hydrogenase in the open field. They showed that field-grown soybeans inoculated with a strain of *Rhizobium* that makes hydrogenase have a greater yield than soybeans inoculated with a strain lacking the enzyme.

Workers at the John Innes Institute in Britain have shown that the hydrogenase made by certain strains of *Rhizobium* is coded for by a single gene and that the gene is found on a plasmid, a loop of DNA separate from the bacterial chromosome. It should be possible to transfer the hydrogenase gene to *Rhizobium* strains that lack the enzyme but have other characteristics making them desirable as nitrogen fixers.

Not all symbiotic nitrogen fixation is conducted by *Rhizobium* bacteria, and not all nitrogen-fixing bacteria attach themselves to legumes. The key to engineering microorganisms that fix nitrogen for cereal crops may be in understanding the details of such natural symbioses. There is only one well-documented instance in which *Rhizobium* has been found to nodulate a plant other than a legume; it was reported by an investigator in Western Australia. A number of other nitrogen-fixing bacteria, however, have been identified and isolated. The bacterium *Frankia alni* fixes nitrogen for the alder tree and certain other nonlegume plants. Alder can therefore be employed in crop rotation much as legumes are: alder seedlings are mixed into forests in order to enrich the soil for commercially valuable trees such as Douglas fir and poplar.

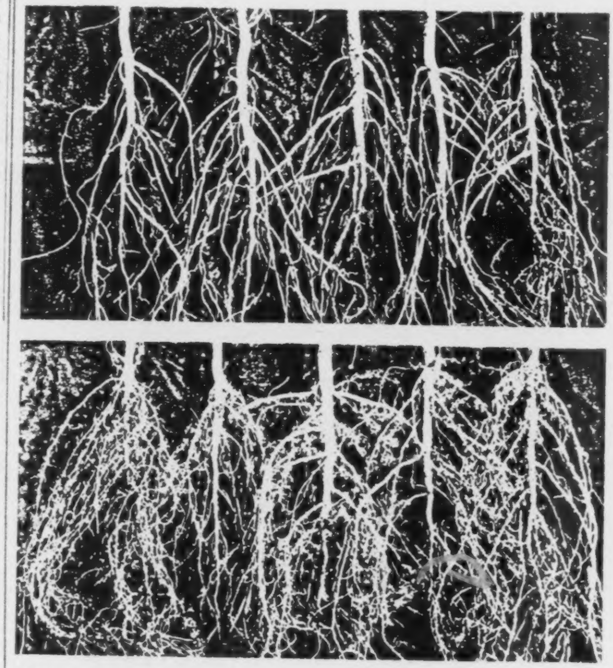
Some bacteria fix nitrogen in the soil without entering into symbiosis with a plant. In my laboratory Stephen W. Ela and I are trying to get the nitrogen-fixing bacterium *Azotobacter vinelandii*, which does not take part in natural symbiotic relations, to bind to the roots of corn and so fertilize the corn. Ordinarily *A. vinelandii* makes no more ammonia than it needs for growth. Hence our first step was to obtain mutants that excrete excess ammonia. This can be accomplished by finding mutants for which feedback pathways that report an excess accumulation of ammonia have been blocked.

To make the ammonia available only to the corn our next goal has been to make the bacterium bind tightly to the roots of the corn plant. Several years ago two of my colleagues transferred certain *Rhizobium* genes to *A. vinelandii* and succeeded in getting the latter to stick tightly to clover roots. If genes that specify binding to corn roots rather than clover roots can be introduced into *A. vinelandii*, the corn should be able to take in the ammonia excreted by the bacteria.

In another aspect of the same project we are attempting to breed varieties of corn that will be able to meet the energy requirements of the bacterium. The corn plants currently under cultivation in the U.S. cannot support *A. vinelandii*. By selective breeding of varieties of corn from throughout the world Ela has been able to increase the production on corn roots of carbon compounds that serve as a source of energy and electrons for nitrogen fixation by *A. vinelandii*. We have now developed corn plants capable of obtaining perhaps 1 percent of their nitrogen from the bacterial association, and we are sufficiently encouraged by our results to try to improve the percentage.

Plants can benefit from many other associations with microorganisms that are just beginning to be understood. At the University of California at Berkeley biologists have shown that the addition of certain strains of the bacterium *Pseudomonas putida* to sugar beet seeds or to potatoes increases the yield of the plant. It appears the bacterium secretes agents that sequester iron in the soil. By this means the iron near the roots of the plant is made unavailable to potentially harmful fungi and bacteria that need iron for growth.

The soil fungi called mycorrhizae colonize plant roots and can create what amounts to an extension of the root system of the plant. For example, they can make phosphate available to plants in phosphate-poor soil by converting the phosphate into a soluble form and transporting it to the roots of the plants. Mycorrhizae can also transport water to the plant, collected beyond the reach of the plant's root system. Plants grown on land reclaimed from strip-mining have been inoculated with strains of mycorrhizae. Other strains may soon have considerable economic importance in forestry because they stimulate the growth of tree seedlings. So far, however, there has been relative-



AUGMENTED ROOT SYSTEM is formed when loblolly pine seedlings are inoculated with the soil fungus *Pisolithus tinctorius*, a species of mycorrhiza. In the upper photograph the fungi are absent and the surface area of the roots is limited. In the lower photograph the mycorrhizae contribute to a denser root system with a larger surface area for the absorption of water and nutrients. The inoculated seedlings grow more rapidly and are more likely to survive. Colonies of mycorrhizae can also extend into regions of the soil not accessible to the root system of a plant, thereby increasing the volume of the soil that can be tapped by the root system.

ly little work done to match specific strains of mycorrhizae to specific plants and growing conditions.

The culture of individual plant cells or groups of cells can provide a source of plant products that is not subject to variability of crop yields or to the uncertainties of international trade. Many plant products, such as pharmaceuticals, pesticides and flavoring agents, have been isolated from tropical plants and can probably be produced by growing plant cells in large fermenters. Moreover, the individual plant cell is an extraordinarily efficient and convenient medium in which to develop new plant varieties. When plant cells are exposed to a mutagen and placed in a stressful environment, varieties adapted to the stress quickly appear and can be selected for culturing.

When a stress such as a toxic agent or the lack of an essential nutrient is imposed, only those mutant cells that by chance are adapted to the stress will survive. In successive generations of cells the progeny of the selected cells can be developed into finely tuned organisms adapted to a specific set of environmental conditions. The same method has long been employed in selecting industrial microorganisms such as molds and bacteria that can synthesize antibiotic agents resistant to degradation.

Once the desired plant cell has been selected it can be grown in culture to form the mass of unorganized tissue called a callus. Plant hormones can sometimes cause the callus to become organized into the stems, roots and other differentiated parts of the mature plant. A plant resistant to the fungal toxin that causes southern corn leaf blight was developed from tissue culture by biologists at the University of Minnesota. They applied the toxin to the culture and then selected the resistant cell lines.

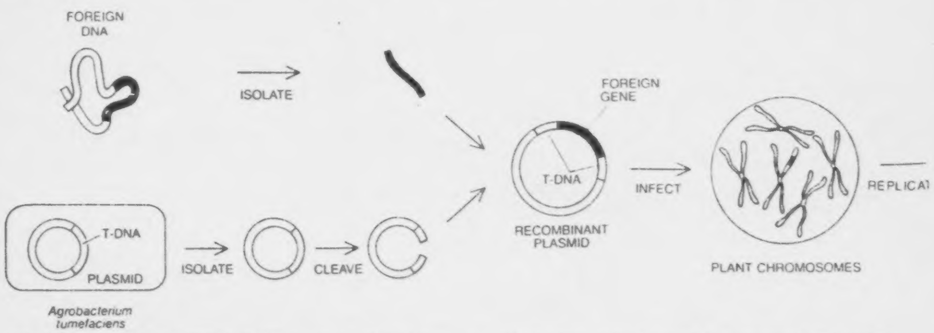
Only a few plants have been successfully regenerated from single cells, and the regenerated plants do not always inherit the properties selected for in the single cell. A herbicide-resistant cell does not necessarily give rise to a herbicide-resistant plant, even though the individual cells remain herbicide-resistant when they are grown again in culture. Moreover, single plant cells grown in culture are usually diploid or polyploid: each cell has at least two copies of every chromosome. The genetic information carried by the cell is therefore coded at least twice. In these circumstances most mutations are recessive. The recessive gene has no effect on the parent plant, but it may make its presence felt in succeeding generations. As a result the characteristics of the progeny of the cells cannot be predicted with certainty. Haploid cells, which have only one copy of each chromosome, have now begun to be cultured, and it should be easier to detect the mutants.

Removing the walls of a plant cell with enzymes gives rise to the naked cell called a protoplast. Protoplasts from two unrelated plants can be made to fuse, creating a single cell that can regenerate a cell wall and grow for many generations in a nutrient solution. In a sense the process is equivalent to sexual reproduction between different species of plants, but a mature hybrid plant is seldom generated. At the Max Planck Institute for Biology in Tübingen protoplasts from a potato and a tomato were fused and did develop into a mature hybrid plant that has been given the name pomato. The pomato plant is sterile, however, and bears neither potatoes in the ground nor tomato fruits hanging from the stems.

The transfer of genes from one organism to another represents the most sophisticated application of microbiological strategies to agriculture, but it is the least well developed of the three I have mentioned. Indeed, compared with the recombinant-DNA methods developed for work with animal genes the work done with plant genes is quite limited. The principles of foreign gene insertion, however, are the same for plant cells as they are for animal cells and for bacterial cells.

In order to insert a plant gene into a bacterium it is first necessary to isolate the gene by means of the enzymes known as restriction endonucleases. The target gene must then be transported inside the host cell by a plasmid or virus vector. In this way workers in a number of laboratories have cloned plant genes in the bacterium *Escherichia coli*. The successful introduction of a foreign gene into a cell, however, does not always mean that the protein product of the gene will be expressed by the cell. For proteins to be expressed additional intracellular chemistry must be set in motion, and it is not yet understood precisely how this can be accomplished in every case.

If microbiologists succeed in expressing plant proteins in bacteria, the proteins can be made by growing the bacteria in fermentation tanks. The process could then be carried one step further, at least in principle. Numerous valuable plant products such as pharmaceuticals, pesticides, oils, waxes and flavoring agents are generally synthesized by plants in multistage chemical reactions where several enzymes take part. If the genes for all the enzymes can be expressed in a bacterium, the bacterium can in effect become a factory for the synthesis of the plant compound.



INTRODUCTION OF FOREIGN DNA into plant cells may be accomplished by exploiting the natural infection process of the bacterium *Agrobacterium tumefaciens*. The bacterium carries a plasmid (a loop of DNA separate from the bacterial chromosome) that causes crown-gall tumors in most dicotyledonous plants and induces the infected plant cells to synthesize the nitrogen compounds called opines. The mechanism of the infection has been termed genetic colonization: a section of the plasmid called T-DNA combines with chromosomal DNA in the nucleus of the plant cell. The plasmid might therefore serve as a vector for inserting foreign DNA into plant cells. The

A much more challenging goal is the introduction of foreign genes into plant cells. Several potential paths are being explored. Perhaps the most promising method focuses on a plasmid found in the bacterium *Agrobacterium tumefaciens*. *A. tumefaciens* induces a tumor called a crown gall in wounded dicotyledons, the large class of flowering plants that includes legumes, tomatoes and numerous other crop plants (but not the cereal grains). The mechanism of the infecting bacterium is to insert a segment of its plasmid into a chromosome of the plant cells. The inserted segment is called transfer DNA, or T-DNA.

The insertion of T-DNA is therefore a natural form of genetic modification, and it endows the infected plant cells with several unusual properties that are probably essential to the formation of a crown gall. Ordinary cells proliferate in culture only in the presence of plant growth hormones, but cells infected with *A. tumefaciens* do not require such hormones. The release of the cells from hormonal control may account for their unusually rapid growth in the tumor. The infected cells also manufacture the enzyme opine synthetase, which catalyzes the synthesis by the plant cell of nitrogen-rich compounds called opines. Opines seem to be required by *A. tumefaciens* as a source of nitrogen. Hence crown galls can be understood as the outcome of a biological strategy developed by the bacterium to secure the nitrogen necessary for its growth.

Investigators at the University of Leiden have been able to infect tobacco cells in culture with *A. tumefaciens*. They showed that tobacco plants regenerated from the infected cells retained the T-DNA and continued to make opine synthetase. More recently investigators at the Max Planck Institute for Plant Breeding in Cologne demonstrated that the gene coding for the expression of opine synthetase is passed on through the seed to succeeding generations. Such results justify some confidence that if foreign genes can be spliced into the *A. tumefaciens* plasmid in association with the T-DNA, they will be expressed as proteins in the mature plant and carried through the seed to the progeny.

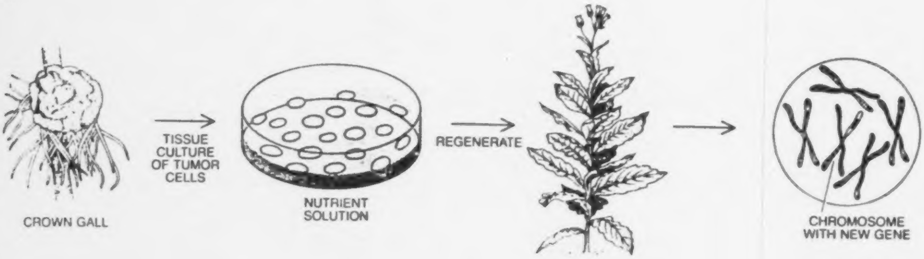
Another method under investigation for introducing foreign DNA into plant cells utilizes the cauliflower mosaic virus (CaMV). DNA from this plant virus can be isolated and spliced into a plasmid for insertion into the bacterium *E. coli*. There the DNA can be amplified, or reproduced many times; the amplified DNA retains the ability to infect the cauliflower and a range of plants related to it. Investigators are now working to determine which places along the viral DNA are suitable for the introduction of foreign genes.

If genes can be introduced into plant cells at will, one important application may be the insertion of nitrogen-fixing genes (*nif* genes) into cereal plants. Workers at the University of Sussex have assembled a bacterial plasmid that includes all 17 of the known *nif* genes from the nitrogen-fixing bacterium *Klebsiella pneumoniae*. When the plasmid was transferred to *E. coli*, the latter, which is normally incapable of fixing nitrogen, became a nitrogen-fixing microorganism.

Even more promising are the recent successes of groups of investigators at Cornell University, the Pasteur Institute and the University of Paris. They introduced the 17 *nif* genes of *K. pneumoniae* into yeast. Yeasts, being eukaryotic organisms whereas bacteria are prokaryotic, are much more closely related to the higher plants than they are to bacteria. Hence the introduction of *nif* genes into yeast cells marks the crossing of a significant biological barrier.

Nevertheless, the yeast cells carrying the *nif* genes were not able to express the inserted DNA: they were not able to fix nitrogen from the atmosphere. The failure illustrates the complexity associated with the genetic engineering of biological functions embodied in more than one gene. The transferred DNA must first be transcribed correctly into RNA by the yeast. Correct transcription cannot be assumed as a matter of course, because the yeast must correctly interpret the bacterial signals to start the transcription and to stop it. The RNA must then be exported from the nucleus and be recognized by the ribosomes as a messenger RNA for translation into protein. The 17 proteins that express the *nif* genes must then function together in the foreign cytoplasm of the yeast cell. There may be impediments to such functioning. For example, the nitrogenase molecule has in its structure a large number of iron atoms. Enough iron is evidently on hand in nitrogen-fixing bacteria, but it is not certain the heavy demand for iron can be met in a plant cell without jeopardizing the synthesis of other enzymes essential to the plant.

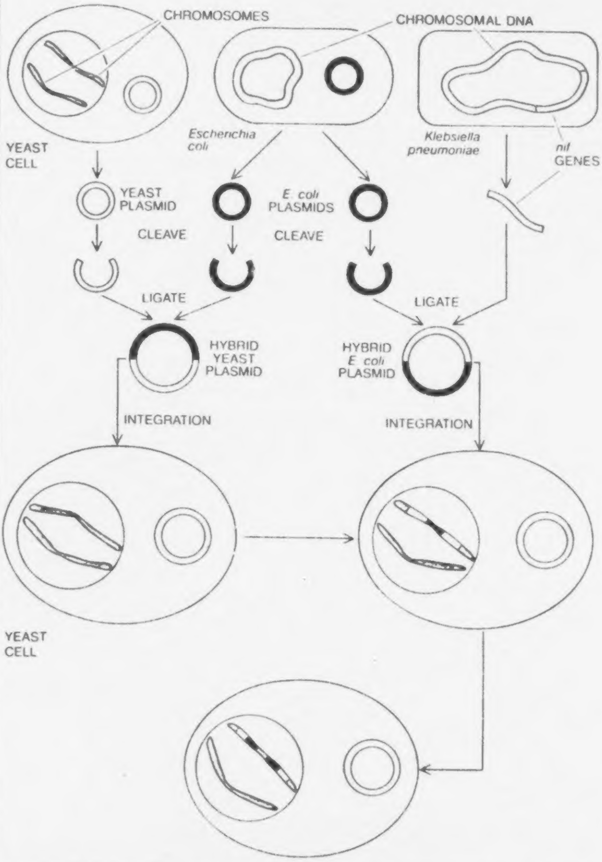
Doubts of this kind can arise even when it seems at first that the expression of a particular plant function calls for only one gene. The enhancement of protein quality in food is a major goal of agricultural research. The stored proteins in some grains and other seeds, for instance, are deficient in amino acids essential to human and animal nutrition. It was once thought that the insertion of a single gene, coding for a stored protein with a better balance of amino acids, would be sufficient to improve the quali-



plasmid would be cut open at a site within the T-DNA and the foreign gene would be spliced into it. The T-DNA is replicated when the tumor cells of a crown gall divide, and tumor cells grown in tissue culture continue to carry T-DNA. It has been possible in some cases to regenerate a plant from the cultured tumor cells; T-DNA is still found in the chromosomes of the regenerated plant. Moreover, the gene carried by T-DNA that codes for the enzyme opine synthetase is passed on to daughter plants as if it were an ordinary dominant gene. If foreign genes inserted into T-DNA are also transmitted to plant progeny, new plant strains could be genetically engineered.

ty of the protein. It is now understood that major seed proteins are often collections of numerous related proteins, each of the latter being encoded by a separate gene. In order to make a significant improvement in the overall quality of the stored protein many of the genes might have to be individually modified.

Although such technical problems may be difficult to overcome, they are probably not the limiting factors in the application of microbiology to agriculture. In the long run more stringent constraints will be the comparatively small financial commitment to basic research in agriculture and the loss of irreplace-



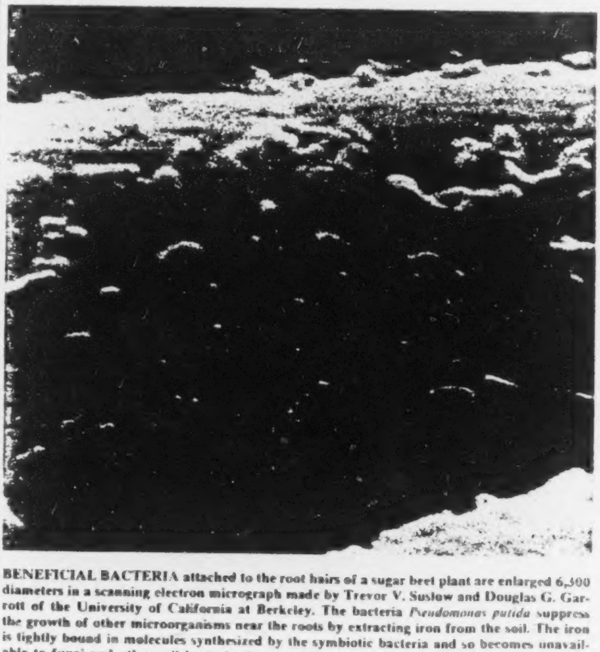
GENES FOR NITROGEN FIXATION have been inserted into the genome of a yeast in a two-stage process. In the first stage plasmids from the bacterium *Escherichia coli* and from a yeast cell are cleaved and then fused to form a single hybrid plasmid. The hybrid plasmid can be recognized by the yeast cell and integrated into its chromosomal DNA. In the second stage the genes to be introduced into the yeast are isolated from the chromosome of the bacterium *Klebsiella pneumoniae*, a nitrogen-fixing organism. The genes, collectively designated *nif*, code for some 17 proteins. Another *E. coli* plasmid is cleaved and the isolated *nif* genes are introduced to form a second hybrid plasmid. Because of the bacterial DNA already inserted into one of the yeast chromosomes the yeast cell recognizes the hybrid *E. coli* plasmid. The plasmid is then integrated into the yeast chromosome. The experiment was carried out by Aladar A. Szalay and his co-workers at Cornell University. Although the insertion of the prokaryotic *nif* genes into the eukaryotic yeast cells demonstrates that genetic material can be transferred between different biological systems, the nitrogen-fixing proteins are not expressed in yeast.

able genetic resources from the total gene pool.

According to an analysis prepared by the National Science Foundation, Federal support for research into all aspects of plant science was $206 million in the fiscal year 1977, 2.3 percent of the $8.8 billion spent by the Federal Government during that year for all basic and applied research. President Reagan's 1982 budget calls for a $691-million research allocation to the Department of Agriculture, a 5.3 percent share of the $13.1 billion now proposed for all basic and applied research in 1982. Of this, however, only a small fraction will be available for studies of agricultural applications of microbiology. For example, the Competitive Research Grants program of the Department of Agriculture will administer $26 million in basic research funds in 1982 for studies of nitrogen fixation, photosynthesis, genetic mechanisms of crop improvement, plant and environmental stress and the requirements of human nutrition. Microbiological studies will receive only $4.8 million. Of all recombinant-DNA investigations monitored by the National Institutes of Health, Federal grants for genetic engineering in agriculture amounted to only $1 million in the fiscal year 1980, compared with some $24.5 million for medical research and $27.5 million for general research.

The accelerated destruction of the gene pool is doubly ironic. It is caused primarily by the clearing of land in the tropical rain forest for farming. Moreover, it is happening at the dawn of an age in which such genetic wealth, until now a relatively inaccessible trust fund, is becoming a currency with high immediate value. Estimates place the annual extinction rate as high as 1,000 species per year, and there is still little organized effort for maintaining genetic reserves.

The microbiologist will be only one worker among many who will participate in the development of new field crops and agricultural practices. It is particularly important that the microbiologist work closely with the plant breeder, not only because of the experience of breeders with the effects of laboratory modifications on field crops but also because many of the techniques of the two disciplines actually overlap. Future work may disclose unexpected barriers to the application of microbiological ideas in agriculture, but it is at least as likely that such ideas can readily be put into practice.



BENEFICIAL BACTERIA attached to the root hairs of a sugar beet plant are enlarged 6,500 diameters in a scanning electron micrograph made by Trevor V. Suslow and Douglas G. Garrott of the University of California at Berkeley. The bacteria *Pseudomonas putida* suppress the growth of other microorganisms near the roots by extracting iron from the soil. The iron is tightly bound in molecules synthesized by the symbiotic bacteria and so becomes unavailable to fungi and other soil bacteria that could be harmful to the plant roots. Competition for essential nutrients is thereby reduced and significantly higher sugar beet yields can result.

THE AUTHORS

ARNOLD L. DEMAIN and NADINE A. SOLOMON ("Industrial Microbiology") are microbiologists at the Massachusetts Institute of Technology. Demain, who is professor of industrial microbiology in the department of nutrition and food science, majored in bacteriology as an undergraduate and graduate student at Michigan State College (now Michigan State University). He then went to the University of California, where he studied at both the Davis and the Berkeley campuses, obtaining his Ph.D. in microbiology in 1954. Before joining the M.I.T. faculty in 1969 he was head of the department of fermentation research at the Merck, Sharp & Dohme Research Laboratories. An active member of the American Society for Microbiology, Demain has served on numerous committees having to do with applied microbiology and fermentation technology. Solomon, who is a research associate in the department of nutrition and food science at M.I.T., is a graduate of the University of Massachusetts. She has worked with Demain since 1972 and in recent years has collaborated with him on a number of journal articles in their field.

HERMAN J. PHAFF ("Industrial Microorganisms") has a joint appointment as professor of food science and technology and professor of bacteriology at the University of California at Davis. A native of the Netherlands, he was trained as a chemical engineer at the Technical University of Delft. He came to the U.S. in 1939 to continue his studies, receiving a Ph.D. from the University of California at Berkeley in 1943. He taught at Berkeley until 1951, when he moved to the Davis campus. A specialist in the ecology and molecular taxonomy of yeasts, he is the coauthor (with Martin W. Miller and Emil M. Mrak) of *The Life of Yeasts*. In addition to Phaff's professional activities he is an accomplished cellist and participates in many musical events on the Davis campus.

DAVID A. HOPWOOD ("The Genetic Programming of Industrial Microorganisms") is John Innes Professor of Genetics at the University of East Anglia. He is also head of the genetics department at the nearby John Innes Institute, a research establishment supported by the British Agricultural Research Council. Hopwood studied natural sciences, specializing in botany, at the University of Cambridge. Soon after his graduation in 1954, he writes, "I began research for my doctorate at Cambridge, studying the genetics of *Streptomyces*, about which little was known at the time. I am still working on the same topic; it gets more fascinating all the time." He obtained a Ph.D. from Cambridge in 1958 and a D.Sc. from the University of Glasgow in 1974. He was a lecturer in genetics at Glasgow from 1961 to 1968, when he took up his present positions. In 1979 Hopwood was elected a Fellow of the Royal Society.

ANTHONY H. ROSE ("The Microbiological Production of Food and Drink") is professor of microbiology at the University of Bath. His degrees, in applied biochemistry, are from the University of Birmingham. After postdoctoral work at Rutgers University and the National Research Council of Canada in Ottawa he taught at Heriot-Watt University in Edinburgh and at the University of Newcastle upon Tyne. He joined the faculty at Bath in 1968. His main research interest for the past decade or so has been in the composition and function of the envelope of *Saccharomyces cerevisiae* (brewer's yeast). This is Rose's fourth article for SCIENTIFIC AMERICAN; the previous ones were "Beer" (June, 1959); "Yeasts" (February, 1960) and "New Penicillins" (March, 1961).

YAIR AHARONOWITZ and GERALD COHEN ("The Microbiological Production of Pharmaceuticals") are microbiologists on the faculty of Tel Aviv University. Aharonowitz got his Ph.D. from Tel Aviv in 1974. He then spent two years as a research associate at the Massachusetts Institute of Technology, where, he writes, "I worked with Arnold L. Demain, who introduced me to the field of industrial microbiology. In Demain's laboratory I studied different aspects of the metabolic regulation of beta-lactam antibiotic production in streptomycetes." Aharonowitz joined the department of microbiology at Tel Aviv in 1976. In addition to his work on biochemical and genetic regulatory mechanisms, he reports, "I am involved in several aspects of the industrial application of biocatalysis." Cohen is a graduate of University College London, after obtaining his B.Sc. degree he took a year off to work on a kibbutz in Israel. He later joined the polymer department of the Weizmann Institute of Science in Rehovot, where he was awarded his doctorate in 1968. He spent the next three years in the U.S. working at the National Institutes of Health before he returned to Israel in 1971 to join the department of microbiology at Tel Aviv. In recent years, he writes, "I have become involved in the area of applied microbiology, and in particular in the ways microorganisms can be exploited to make useful amounts of vitamins, amino acids and antibiotics."

DOUGLAS E. EVELEIGH ("The Microbiological Production of Industrial Chemicals") is professor of microbiology at Cook College/New Jersey Agricultural Experiment Station, a division of Rutgers University. Born and educated in Britain, he received his Ph.D. from the University of Exeter in 1959. Before joining the Rutgers faculty in 1970 he worked for six years at the Canadian National Research Council's Prairie Regional Laboratory in Saskatoon. In his spare time, Eveleigh writes, he "rejuvenates on table tennis and legerdemain."

ELMER L. GADEN, JR. ("Production Methods in Industrial Microbiology"), is Wills Johnson Professor of Chemical Engineering at the University of Virginia. His degrees are from Columbia University: B.S., 1944; M.S., 1947; Ph.D., 1949. He joined the faculty at Columbia soon after obtaining his doctorate and taught there until 1974, when he left to become dean of the College of Engineering, Mathematics and Business Administration at the University of Vermont. He was appointed to his present position in 1979. Gaden's primary professional interest is in the development of biochemical processes for the production of food and chemicals and for the disposal of wastes. In 1970 he was the first recipient of the Food and Bioengineering Award of the American Institute of Chemical Engineers. In recent years, Gaden reports, he has been particularly active in the work of the Board of Science and Technology for International Development of the National Academy of Sciences/National Research Council.

WINSTON J. BRILL ("Agricultural Microbiology") is Vilas Research Professor of Bacteriology at the University of Wisconsin at Madison. A graduate of Rutgers University, he got his Ph.D. in microbiology from the University of Illinois at Urbana-Champaign in 1965. After two years of postdoctoral work on the genetics and regulation of amino acid metabolism at the Massachusetts Institute of Technology he moved to Wisconsin, where his research is devoted primarily to the study of the biochemistry, genetics and physiology of nitrogen fixation [see "Biological Nitrogen Fixation," by Winston J. Brill; SCIENTIFIC AMERICAN, March, 1977]. In addition to his academic work he has just been made director of research for the Cetus Madison Corporation, a newly formed subsidiary of the Cetus Corporation that will concentrate on the application of modern microbiological techniques to agriculture.

Mr. GORE. Thank you very much. Your testimony raises numerous questions but we will hold them until the other members of the panel have presented their statements.

Dr. Mott from the Department of Botany at North Carolina State University, we are delighted to have you. Please proceed.

STATEMENT OF DR. RALPH MOTT, DEPARTMENT OF BOTANY, NORTH CAROLINA STATE UNIVERSITY, RALEIGH, N.C.

Dr. MOTT. Thank you.

Due to difficulty with the mails, I have been given permission to submit my written statement later.

Mr. GORE. That will be fine.

Dr. MOTT. I am with the Department of Botany at North Carolina State University. I will approach biotechnology from a little different point of view having to do with tissue culture. That is my background and expertise. I was trained in dealing with the essential totapotency of plant cells. That word indicates that one can take a cell from a higher plant, isolate it, and from that cell get back to the whole plant, i.e., generate a whole plant from a single cell.

My interest and expertise is around the area of how to manipulate the sterile and defined nutrient media which bring about those changes, whether through embryogenesis where a cell divides in an orderly way to form the whole embryo or through organogenesis where a cell divides first to form a mass of cells called a callus and then afterward, from the callus, a shoot develops. Subsequently the shoot may be rooted and we end up with a whole plant.

Now you say, OK, what does all that have to do with biotechnology? Tissue culture interacts and impacts upon the broad biotechnology in three ways. First, it is a means of propagating plants with economy in both time and space. Second, it is also a means of providing the cells in culture, in a form where one can deal with them in terms of inserting genes or recombinant DNA products into those cells, and later regenerate a plant from the altered cells. Since plants can be regenerated, and cloned, it can provide the vehicle by which genetic engineering is distributed to the farmer. You genetically engineer cells to achieve one plant. Then to get from that one plant to many plants or to many seeds, to get this special plant out into the field and make some impact economically, tissue culture propagation is viewed as a good prospect for the vehicle to disseminate these genetic engineering tricks as improved planting stock.

The third place it impacts is that, since one can deal with many cells in a small container in the laboratory, you have the opportunity of selecting one good cell among millions of candidate cells. Then presumably you could take that good cell, produce a plant from it, you can see the efficiency of this route compared to the alternative, which is to assemble a million plants in a large field, then select a good plant out of those million plants. If one can do the selection in a test tube, then one enters into a whole new realm of efficiency that we have not seen in agriculture so far. That is probably the biggest impact of tissue culture.

Among these three areas of impact the propagation, the supplying cells for genetic engineering which can regenerate plants, and the ability to select desirable cells among many—the first two require tissue culture techniques which will be true to type. If you wish to propagate a plant, you would like the propagules of that plant to be like the parent plant and not to change. That would be good to disseminate planting stock from a genetically engineered cell, for instance. You would like the plants to stay like you made them. The thrust in this application of research is to maintain true to type in the propagation.

On the other hand, if you are dealing with cells and selection for desirable mutants, the tissue culture approach offers you yet another approach which is just the backward of that. Tissue culture can generate variability. Handled properly, the tissue culture methodology allows that the cells placed in culture will change and assume new characteristics, much like mutations. This is a new area, a new kind, possibly a brandnew kind of genetic variability, available within crops such as potato and sugar cane. Apparently, new kinds of variability arise from the tissue culture manipulation. On the one hand, if you were trying to propagate an improved crop you would cry and moan because you now have variability coming in where you did not want it. In the right place and under control, and as an adjunct to recombinant DNA technology, the variability may be just what we are after for crop improvement. So this tissue culture approach has both sides involved in it.

Plant tissue culture has great potential along two distinct avenues of biotechnology. Tissue culture has advantages all by itself, and it has even greater potential when married up with the ability to manipulate the genes through recombinant DNA technology.

I would like to address first the potentials of tissue culture as a science by iteslf, as a tool by itself. Tissue culture has been known for about 30 years. The ability to propagate plants by tissue culture has been known, say, for 20 years. It has just been in about the last 10 years that it has had significant impact, and that has been in the horticultural industry. Many plants that you would buy for ornamental purposes or plants in the house now have tissue culture in their background. The reason being that methods have been devised to propagate, economically, plants which are true to type. The tissue cultured stock material may be kept in a refrigerator rather than as plants in the large greenhouses. The cultured plants are generally disease-free. Stock culture can be selected from a plant which has a particular flower color or a particular aspect which makes it commercially valuable because it is rare. From such stock cultures one can generate large numbers of identical plants rather quickly.

By following the development of this technology, we have an opportunity to see how long it takes for such technology to reach commercial use. It has taken about 10 years. The methods are considered rather trivial by most people who work with tissue culture. Since it has had a commercial impact in the horticultural industry, now there is much more attention paid to it. Suddenly something which was trivial and not very exciting scientifically, and which certainly did not gain much recognition when published in the journals, now becomes what many of the journals are about. The applied journals now have many reports that a new species can be tissue-cultured in such and such a way for propagation.

This kind of technology was available for some time, but it took a commercial use before it became fashionable, you might say, for many people to work on it. It is just surprising, as you look over the literature, how widely that has expanded in just the last few years.

Even more potential exists in the integration area where we bring tissue culture and recombinant DNA together and marry them up for crop improvement. I am involved in a program having to do with pine trees and increasing forest yield. That program at present is focused on mass vegetative production of propagules by tissue culture. We wish to go into the field, select the biggest, tallest, best pine tree we can find and propagate it as planting stock. There would be significant economic impact by just doing that simple thing which has no recombinant DNA technology in it at all.

When the methodologies are worked out for doing that, and we are well along the way now, then the next thing would be to genetically improve even the best trees. Since pine and forest conifers as a group are not well-defined genetically and do not have strong breeding programs such as we take for granted in corn and tomato, we expect to play catch-up with conifer trees by applying the recombinant DNA technology with the tissue culture vehicle.

What is needed for that expectation to come about? One must be able to regenerate the cells back into a plant. One must be able to be reasonably sure that the traits expressed as a cell in culture will be also the traits expressed in the plant, and you have to have some reasonable assurance that the traits will be transmissable through subsequent generations, say, in a breeding program.

Where are the obstacles at percent? The first is the inability to handle forest species for tissue culture regeneration. In terms of regeneration, you can list the crops which can be regenerated now from a single cell. They are few but the list is expanding all the time. It takes just plain hard work at the moment, very methodical and painstaking, laborious research of trying experiment after experiment without much by way of a guide in terms of dogma or theory. Making a new variety behave in culture just takes a whole lot of time.

There are two approaches to a solution. One is, you could apply more people doing that sort of research and you could advance further. Another approach is to do the basic science as Dr. Brill mentioned; the basic science which would allow us to come up with some dogma or some theory, this would cut down that cut-and-try kind of experimentation.

A second obstacle is in understanding and making use of the traits expressed by the cell in culture. That has a many-pronged approach. We wish to select in culture for a trait desired in the plant. Right now, the traits which could be used reasonably soon would be a desirable trait in the plant which could logically be expected to be expressed by all of its cells, such as resistance to cold or resisitance to salt; something like that. Other things, such as making the leaves a little better, making the flower a little different color, making the corn grain have a different protein, are not expressed by all of the cells of the plant and we simply do not know how to manipulate the cells in culture so that they will express leaf genes or grain genes. We have to take the seemingly random array of expressed genes as they stand in cultured cells.

I would think it is going to take some major breakthroughs in that area to get to a place where we can reliably have selected traits of cultured cells carried on to the whole plant. In that respect, a breakthrough would open up several things. One would be the ability to select among a million cells for a trait of importance, with assurance that the trait would be expressed in the regenerated plant. It would also bring us to another area of impact where the cultured cells could be made to express certain biochemical traits. Cell cultures could then be used to generate biochemicals much like the fermentation industry does with bacteria at the moment.

Therefore, sort of in wrap-up, I would say we have in the agricultural land grant schools strong breeding programs and strong crop management programs. These are not as yet well integrated with this emerging biotechnology. Even such simple things as using tissue cultures to generate large numbers of plants to help the breeding programs are difficult to impliment because of the research risk involved. It usually does not work at first try on an agricultural crop. Consequently, the money that comes through the university systems and through the granting agencies to support such research is for applied programs, but fundamental research must yet be done. On the other hand, with typical granting agencies we who attempt to work with crop plants are continually in competition with those types of experiments which, from a theoretical point of view, can usually be done better on an animal system because so much more fundamental science is known about those systems. From the practical system, coming through USDA and other funding agencies more attuned to the plant side, there is a bit too much of a risk with tissue culture and recombinant DNA compared with what can be done with a classical breeding program. In the universities, we are sort of caught in a never-never land in between. It is the industries now who are seeing a commercial impact in this system. They are feeding the pump a little bit and that is where the money is coming through now for the type of research which spans those two areas, the very basic and on to the applied.

My written comments will touch and expand upon these points. Thank you.

[The prepared statement of Dr. Mott follows:]

STATEMENT BEFORE THE SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT,
OF THE COMMITTEE ON SCIENCE AND TECHNOLOGY,
OF THE U. S. HOUSE OF REPRESENTATIVES

June 9, 1982

Recombinant DNA Techniques and Applications to Agricultural Sciences

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Dept. of Botany, N. C. State Univ.
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This morning I will direct attention to biotechnology as applied to improvement of crop plants and the role of plant tissue culture in this special application. Plants are made up of many cells, each functioning in a coordinated way to comprise the plant, and this sets plants apart from the single-celled bacteria and yeasts where recombinant DNA methods have already made significant impact, as in the fermentation industry.

My research experience lies in the area of plant tissue culture. My laboratory in the Dept. of Botany, N. C. State University, has been concerned for the past ten years with the regeneration of plants from cultured cells. Cultured cells are totipotent in that roots, shoots, or the whole plant may be regenerated from them by suitable manipulation of the sterile, nutritive medium on which cells are cultured. Although suitable manipulation recipes exist for some species, many crop species do not regenerate plants. We seek to discover fundamental biological aspects of the process and thereby expand the list of successful crop species. We currently have a major effort toward tissue culture propagation and improvement of forest pine trees.

The nuclear genetic material of microorganisms, plants, and animals consists of DNA. Recombinant DNA techniques allow selected portions of this DNA to be transferred from cell to cell. The transferred DNA can function in the foreign cell; e.g., animal and plant genes have been demonstrated to function in bacterial and yeast cells. This technology has already been used to modify microorganisms to produce insulin, interferon and growth hormones. The microorganisms grow as single cells in fermentation culture vats and produce the desired chemical as a byproduct. We can expect rapid progress in this field, but crop plants are made up of many different cells, each using only a portion of its genes in coordination with other cells. This and the fact that plant genes are carried in complex packages called chromosomes make it much more difficult to insert foreign genes in the right place on the right chromosome and under control for expression in the right cells of a plant.

Plants may benefit from gene insertion into the fungal mycorrhizae which aid plant roots in their nutrient and water uptake. Likewise, research on plants will benefit from availability of specific chemicals produced by genetically engineered bacteria or from antibodies produced through hybridoma monoclonal antibodies, etc. However, genetic engineering of the plant itself is and will be impeded by our lack of understanding with respect to cell to cell coordination and the chromosome structure and gene control. It is in this area where tissue culture offers advantage. The cells which comprise a plant can be separated and grown individually and even caused subsequently to engage again in cell-to-cell coordination to grow into an entire plant.

Plant tissue culture is part of the biotechnology in agriculture in three usage categories:

Usage 1. Faithful clonal propagation of plants in large numbers from selected superior plants.

Usage 2. Production of suitable individual cells on which laboratory recombinant DNA methodology can be applied. A necessary adjunct is the requirement that cloned plants be regenerated from such altered cells. It is the improved plants and their seed which ultimately reach the farmer.

Usage 3. Production of cultured cell populations, millions of cells, from which cells with desirable traits may be selected. If cell traits reflect desirable plant traits and selected cells

> can be made to regenerate plants, then great efficiency results from this indirect selection for desirable plants. Cells with special properties may also be grown as cells in continuous cell culture for the production of useful plant biochemical byproducts. The desirable plant or cell traits may be resident in the initial population or induced through recombinant DNA or other methods.

The tissue culture procedure itself, without recombinant DNA involvement, opens several doors to crop improvement.

Propagation of large numbers of plants "true to type" with the parent plant (usage 1) has considerable value to the horticultural industry. Stock cultures, stored in refrigerators, can be used to regenerate a million plants in a few months--all of the same genotype and, with suitable care, all disease-free. A plant with desired genes may be propagated indefinitely in this way. Currently there is less use of this propagation for field crops because many crops must be planted from seed rather than as young plants. A more important problem is that the existing tissue culture methods do not apply for most crop plants. Forest tree species such as pines and most conifers are increasingly being treated as a crop for fiber and for a renewable energy and chemical stock resource. The trees are planted as seedlings but traditional vegetative propagation of the superior trees by rooted cuttings is not possible. The application of the tissue culture propagation methods as used in horticulture would be of great benefit to improve forest yield, but the methods do not work for these trees. We have had success with forest trees, and we are continuing the work to achieve this early pay-off.

Two general points are thus evident. First, biotechnology for some crops might involve just the modification and application of existing methods to that crop. Second, methods for one crop are not generally transferrable to other crop species and much hard work is necessary to discover new methods. The discovery would be quicker and more reliable if we understood more about the processes involved as cells coordinate their activity to form a plant.

The tissue culture process is also capable (in unknown ways) of generating variation in the plants produced. Superior plants can be selected from hundreds of such variants, resulting in an improved planting stock. If the selection could be made among cells in a culture (usage 3) rather than among plants in a field, the efficiency of selection can easily be one in millions. Selection among tissue-culture-produced plants has been used for potato, and among cultured cells for sugar cane, for example. The major problem is learning to culture cells so that the cells show selectable genetic traits which will be useful in the whole plant. Real progress awaits a breakthrough in understanding of the control of specific gene expression by cells as they grow in culture.

Cells in culture can be turned into protoplasts by enzymatically removing their cell walls. These living, naked plant cells can fuse together with absence of their covering walls, and when they do, their individual chromosome complements become mixed in the resulting fused protoplast. The combined sets of genes may be stable in the new fused cell, or chromosomes may be lost during subsequent cell divisions after a new cell wall has regenerated. In either case, a stable genetic configuration can eventually be reached in the dividing cells, which may have some genes from both parent protoplasts. The outcome of this somatic hybridization is similar to that which occurs in normal sexual crosses involving sperm and egg in flowers. An important difference exists in that with protoplasts, crosses can be made between plants that normally do not cross. A wider range of genes is thus made available for use in a crop plant. Furthermore, if genetic traits can be selected in cell culture (usage 3), only those cell fusions resulting in desirable traits need be recovered to plants.

It is evident that tissue culture itself is an important part of the biotechnology which can be applied to crop improvement. The recombinant DNA technology can be applied at the cultured cell level (usage 2) to achieve select gene insertion. Recombinant DNA methods can also be used to characterize more fully the gene complement available for selection and to investigate how the individual genes interact. This combination of both tissue culture and recombinant

DNA approaches is the area of biotechnology which holds the most promise for crop improvement. It is also the area of least realization of that promise, as yet. In our limited grasp of the cell's genetic workings, it seems that all things may be possible. Indeed, the news media would have us believe that it will be so. As we learn more, restrictions and complications arise, and it must be our mission to evaluate and codify this new area for multicellular plants and turn it to useful ends.

A Cooperative State Research Service southern regional project, "Molecular and Cellular Genetics for Crop Improvement," recognizes the distinct potential for tissue culture methods for plant improvement. These methods are likely to have more early achievements than will foreign DNA manipulations, since most of our knowledge of genes in crop plants relate to the phenotype of the plant; a better grain, a taller shoot, a broader leaf, etc. Little is known of the molecular intricacies underlying these responses, and in most cases the responses are the result of not one, but several genes. Tissue culture offers a way to select for even multigenic plant phenotypes and also a way to investigate the cellular and molecular participations in plant response, because the cells may be cultured independently. This is an important point and one that affects our view of the future. Whether by tissue culture or by recombinant DNA, plant improvements will have to be tested against the realities of field performance and marketability. Those scientists who deal with these aspects must be considered vital to progress with biotechnology. They know of environmental effects and of genetics, but in the language of statistics, rather than molecules. This knowledge must serve as a guide in the quest for cellular and molecular understanding.

The rudiments of plant tissue culture have been known since the early forties. In the last twenty years it has been possible to propagate certain plants by tissue culture, but only during the last ten years or so have these methods had significant commercial impact in the horticultural industry. It appears that one can expect about a 10-year lag between discovery and applied use. More advancements will come as the methods are brought into use for agricultural crops.

I believe that there are a variety of laboratory methods now available, which need only be modified and put to applied use. The major difficulty is one of establishing meaningful dialog between the laboratory scientist and the field-oriented scientist, so that the solutions can be matched up with appropriate problems. This is not as easy as it sounds, since each science has its own traditions, its own approach and accepted ways of doing things. We must seek ways of fostering this dialog, realizing that it usually falls between the cracks of customary funding patterns. The bench scientist is normally encouraged to discover new things, not polish and modify the old. The field scientist feels pressure to improve the crop, and proven methods are more easily justified than some new, risky method which may not work. Success requires the input of both scientists, and we should be alert to opportunities to foster collaboration in the long term so that discoveries are put to good use, and so that applied needs can lead to useful discoveries--a time-worn problem, but one that is upon us with new urgency.

We are coming upon another dilemma which is part of the biotechnology. Both tissue culture and recombinant DNA methods offer new ways to improve crops. They also offer new ways to gain fundamental biological information about how cells work, and we need that information to further develop methods useful for crop improvement. What gets in the way is the time scale. We are finding that we know too little about basic cellular processes and about how cells communicate and coordinate their activity to grow an entire functioning plant. We need this information now; the lack of it is holding us back in the development of biotechnology. Yet that information-gathering job seems too big and too diverse. A major commitment to gathering this information seems at odds, almost a step backwards, with the glamour of the futuristic view of biotechnology.

In our haste to get on with the developing potential of biotechnology, I think we must be cautious to avoid pushing a disproportionate effort along this glamour line to the exclusion of good plant science in other allied areas. After all, it seems that we as a nation have fostered those sciences which were necessary to what is now considered traditional breeding and crop management programs.

We were successful. We have developed a strong agricultural productivity base. We now find that certain areas critical to the new biotechnology were neglected. These areas of cellular function, cell communication, and basic physiology and molecular genetics were not so important to traditional crop improvement programs. If we again succumb to a narrow goal-oriented plan this time around, what important things for the future will we leave out? I urge that we seek breadth and balance as we move to redress the perceived inadequacies of the past.

We will continue to see advancements in gene manipulations with microorganisms in the short term. There is a gap between the field notion of genes and the molecular behavior, and a gap between understanding single-celled microorganisms and multicellular plants. These gaps will hinder direct molecular gene manipulations on crop plants in general, although we may see spotty advancement as some very special circumstances are recognized and utilized. The elimination of these gaps will require a major infusion of support to the plant sciences by this country, building on the base we have. Our current success leads to grand expectations, and these expectations can be met if we invest broadly in the research effort. New biological tools are available that have never before been available to man. We must gain experience with them and gain the necessary fundamental understanding to appreciate their potential. We must provide the support and incentive to keep people at the university laboratories to train the scientists that are needed. We must also recognize the need to foster collaboration and interaction between sciences and specialties on a broader scale and perhaps more directly than has been done. Movement towards these ends must be done immediately if we are to retain our base and resume a leadership role in this rapidly advancing field.

Mr. Gore. Well, both of our first two witnesses have touched upon the university-corporation relationship and how it is changing in this area and the pressures leading to that. We are going to touch on that in questions. We have another 2 days of hearings coming up later this month on precisely that issue but we will explore it a little bit in questions today.

Dr. Wood, we have a vote on the floor and it occurs to me that it might be better to avoid interrupting your statement in the middle and instead take about a 5, 6, 7 minute break right now. Then when we come back we will start with your statement.

[Recess taken.]

Mr. Gore. The subcommittee will come back to order. Dr. Wood, we were just at the point of receiving your testimony. Let me again welcome you in behalf of the chairman and tell you that he wanted very much to be here himself but he is chairing a hearing of the full committee with Bell Labs on the future of Bell Labs as a result of pending legislation that will change the revenue sources for that great national resource. Otherwise he would have been here, and he was frustrated by his inability to be in two places at once. I know we have all had that feeling.

Please go ahead with your testimony. We are anxious to hear from you.

STATEMENT OF DR. F. A. WOOD, DEAN FOR RESEARCH, INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES, UNIVERSITY OF FLORIDA

Dr. Wood. Very good. My name is F. A. Wood. I am dean for research, Institute of Food and Agricultural Sciences at the University of Florida, responsible for our statewide agricultural research program which, with Federal and State funding including grants, is about a $57 million agricultural research program.

I will pick up on some of the comments that Drs. Brill and Mott have made this morning, but I will not say much more about the importance and significance of these technologies and techniques to agriculture. I would like to outline what I think the manpower situation is, give you a few statistics, and suggest a possible solution or two to a very critical problem facing this country at the present time.

A point I would make—at least this is how I look at the genetic engineering-biotechnology area—is that the techniques, the cellular and molecular genetics techniques, the gene-splicing techniques, the protoplast generation of plants—both areas have been described here very well this morning—I look at these as research techniques or research technologies and the product of these, the modified organisms, for example, the bacteria, the plant, the animal, whatever, as the biotechnology.

It is important, I think, when looking to the impact of biotechnology in agriculture to look at not just the recombinant DNA or the genetic engineering component as so many people do. I think we must include activities such as protoplast generation and in the area of animals, the hybridoma-monoclonal antibody systems, and related techniques should be considered. As I talk this morning I will be speaking about the full spectrum of these kinds of activities with respect to agriculture.

One of the needs in agriculture would be in the area of the identification of genes. We know a lot of genes exist but we really have not identified, in all the years we have been working with plants and animals, that many genes. In addition, we know the chemical description of many, many few genes, so the identification and the chemical description of genes are two areas that need additional effort in if we are going to realize some of the potential impacts that have been alluded to this morning.

Also the development of vectors: How do we transfer genes from where they exist in nature, in cropping systems, microorganisms, and so on, how do we transfer these genes from that setting to where we want them? There are a number of different techniques and possibilities but we have a long way to go in terms of developing efficient vectors for the movement of genes from plant to plant.

As far as impacts in agriculture, you can just let your mind run and about anything you can imagine, I think, probably will happen in the next 10, 15, 20, 25 years, some time down the road, and I do not think that is being frivolous in any sense. When you consider that genetic material, the nuclear material of all living organisms, is similar and that we have been able to move genes from animals to bacteria, for example—quite a transition, and have those genes survive and function in that new environment, then there is really probably an opportunity to move, at some point in time, just about any gene or group of genes just about anywhere we would like to.

An area of particular interest and related to energy is the development of biomass, biomass for fuel. There is a lot of interest in this in the subtropical and tropical portions of the United States. We are very much interested in this in Florida and have a rather extensive biomass production program; it involves the production of a set of energy crops. We will produce these new energy crops using the traditional techniques but there is tremendous opportunity for applying the new techniques in cellular and molecular genetics as well, and we are moving to do this.

They have application in the actual breeding and development of individual plants in the degradation or breakdown of biomass into a usable form and in the conversion of cellulose; there is a lot of potential for genetic engineering of the microorganisms involved and it needs to be done. Once the cellulose is broken into simpler carbohydrates, there is an opportunity to increase the efficiency of the methane bacteria in converting the carbohydrates to methane. Yes, there are tremendous opportunities and potential for the application of these techniques in the development of energy crops, nonfood kinds of energy crops.

Another area that we are excited about and others are is in the root associations. In addition to nitrogen-fixation that has been mentioned by Dr. Brill this morning, the mycorrhizae represent a fungus-root association that occurs in most living plants. This fungus-root combination enhances the efficiency of the plant in the uptake of water and certain kinds of nutrients. There is an opportunity to increase the efficiency of plants as they utilize water and nutrients by modifying the mycorrhizal system with these techniques.

In terms of a time frame, I would definitely agree with the earlier comments. Things are moving much more rapidly than I think we appreciate but I still think it is out there 5, 10, 15, 20 years before the maximum impact will be felt. In the meantime, a lot of things will happen in the next 5 years.

The manpower question, a little background on that: First of all we have a general problem in the area of science as far as manpower needs in the United States are concerned. I received some numbers just a few weeks ago indicating that in the area of the biotechnologies, the production of Ph. D.'s in the United States for 1981 or thereabouts was approximately 100. By contrast, Japan produced somewhere between 600 and 800; this is a development that I do not like.

There has been an evaluation of the need for scientists in agriculture in general. This has been completed by the RICOP Committee of the Division of Agriculture of the Land-Grant Association. They found an overall 15 percent shortfall in manpower in agricultural research in general, research and education, and there are similar kinds of statistics in other areas.

As a kind of backdrop, when you look at biotechnology and at what has happened, that is, the development of approximately 150 new biotechnology firms in the last 3 years, expansions in the research programs of already existant firms, the move to expand research and education programs in molecular and cellular biology by universities and colleges in the country, yes, there is a shortage of manpower. There is a very definite shortage. I do not known the magnitude. I wish I did. In fact, I am involved with the Division of Agriculture Committee of the Land-Grant Association—the fact is, I am the Chairman of it—to get at this, among other issues relating to this particular area of research and education. We will be making a report to the Land-Grant Association next November.

There are some special problems as you look at this shortage as far as the universities are concerned. First of all, industries have traditionally and are now paying higher salaries, so they are attractive. Now I would hasten to add that not all scientists are attracted by dollars; there are many scientists interested in working in the academic environment, working in research in that kind of setting, working with graduate students and so on, so there are other factors. However, the fact of the matter is that a doubling of salary, which has happened in some instances, is difficult to deal with.

There has also generally been a reduction in research and development for agricultural activities at the Federal level and in most instances at the State level. In fact, we are working with funding levels equivalent to those of the last sixties. One of the sad consequences of this decline in effort and the situation that the academic institutions find themselves in is that it is in that setting that the production of new scientists occurs. The balance of my comments will be a brief outline of what I think is a partial solution to this problem.

We need two things: We need additional research in the agricultural sciences in the area of the new biotechnologies, and we need additional scientists across the board in this area in particular. I think the most straightforward and readily available solution is that of simply enhancing support for research and education programs of the land-grant universities. An increase in research program support can accomplish both: It will not only increase the amount of activity in research but since graduate students can be and often are involved, it would increase the output of new scientists.

The land-grant universities, in a nutshell, have the mission which comes out of their legislative mandate of the mid-1860's. They have the infrastructure for graduate education programs. They have the liberal arts and sciences, the medical schools, and the college of agriculture and in many instances on the same campus. They have a track record, an excellent track record in basic and applied research, and this track record has been in large part, along with activities in the Federal and private sectors, responsible for the tremendously successful high technology agriculture we have in this country.

Most of the agricultural scientists in the United States are produced by the land-grant system, not all but most of them. It is also important to realize the land-grant universities house the Agricultural Equipment Stations that direct the agricultural research in the public sector in the States.

A few comments about the Agricultural Experiment Stations that I think are germane: There are about 14,000 scientists involved in the agricultural research programs of the Experiment Stations in the States. Their annual research budget is about $1 billion. The physical plant these scientists reside in—the scientists, their technicians and graduate students—is valued at at least $8 billion, so we have a tremendous resource that has been put in place by the taxpayers of this country.

Of these 14,000 scientists, approximately 5 to 10 percent are involved in the kind of research that we have been talking about here this morning. I cannot give you an exact figure. Hopefully in 6 months or so we will have that but I would say 5 to 10 percent at the outside are involved in research and education in this area.

Very simply—and I know these are times when budget increases are difficult to obtain—very simply, we need increases for agricultural research and graduate education. There is a variety of mechanisms. The formula would be one, the competitive grants program another. I think both should be carefully examined. The kinds of research that we are talking about in many instances requires a long-term commitment. This can best be provided by the long-term formula kind of fund support.

In contrast, we need a mix of scientists and a continual change and redirection of elements of the program. Expansion of the competitive grants program, and I mean a significant expansion of that program, could provide for these needs. It also brings non-land-grant universities into the agricultural research arena, which is a darn important aspect.

Currently, there are efforts to increase our scientific manpower output. The Resident Instruction Committee on Policy of the Division of Agriculture of the Land-Grant Universities has proposed that a $12.4 million program be initiated this coming year; that the $12.4 million be used for support of graduate students doing research in agriculture in general. This would provide support for about 650 to 700 graduate students and the funds would be provided to the States or the institutions on a competitive basis.

In summary, the new molecular and cellular genetics techniques in the general field of biotechnology are going to have a fantastic positive effect or impact on agriculture. We do have a significant manpower shortage, a very significant and critical manpower shortage. If we are going to capitalize on the investments that have been made and some of this potential, bring it into reality, we definitely need to move and we need to move now. I think there are existing mechanisms within the confines of the land-grant system, as I have indicated, that make it possible for us to maximize our output or, said another way, take advantage of the investment we have already made.

[The prepared statement of Dr. Wood follows:]

PRESENTATION TO THE SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT OF THE COMMITTEE ON SCIENCE AND TECHNOLOGY OF THE HOUSE OF REPRESENTATIVES

RECOMBINANT DNA TECHNIQUES AND APPLICATIONS TO AGRICULTURAL SCIENCES

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Dean for Research
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My presentation this morning will focus on two different but related topics: the impact of the new techniques in molecular and cellular genetics and their impact on agriculture, and the shortage of talent at the scientist and technician levels to fully exploit opportunities that these recent advances in molecular and cellular genetics have provided.

Biotechnology has had a significant, positive impact on agricultural production since the founding of the System of Land-Grant Universities in the mid 1800's and the inception of organized agricultural research in this country. Biotechnologies in the form of improved plants and animals, methods of pest control and cultural practices are legend today. In fact, every variety and/or species of plant and every breed and crossbreed of animal under management in agriculture today has been improved in one way or another. Examples of specific biotechnological advances include wheats and other small grains that yield 10, 15, and even 20 times the amount of grain their ancestors produced; soybean varieties adapted to a particular photoperiodic regime and photoperiodic ecotypes modified with specific pest resistances such as the new variety Foster with genes for resistance to each of three different nematodes; tomato varieties that thrive in pest-ridden environments and mature through a relatively short period of time to enhance harvesting, beef cattle breeds and crossbreeds adapted to perform efficiently in the northern plains, the high plains of the Southwest, and the Gulf Coastal Region, and breeds of swine and poultry that perform efficiently in crowded conditions leading a totally confined life from birth to slaughter. These are but a few of the many achievements that have been accomplished through the development of biotechnologies in agriculture.

New molecular and cellular genetics techniques have evolved during the last 20 to 25 years and are available for application to the solution of problems confronting agricultural production. These techniques include recombinant DNA methods, protoplast generation and somatic hybridization of plants, and hybridoma-monoclonal antibody methods. Recombinant DNA techniques provide a basis for directed genetic improvement of plants and animals. In the past, genetic improvement of plants has been associated with chance, i.e., when a scientist transfered pollen from the male flower to the female flower he or she did not exercise control over what genes would end up in what particular members of the progeny. Recombinant DNA techniques provide the potential to place a given gene in a given plant or microorganism.

Accomplishments in recombinant DNA research suggest that genes have the capacity to function in a variety of genome and protoplast environments; for example, animal genes have been demonstrated to function in bacterial cells. Also, the genes of higher plants have been demonstrated to function in bacterial cells. Since the nuclear genetic material of both plants and animals consists of deoxyribonucleic acid (DNA) it is evident that the potential exists to transfer any gene or group of genes from one living organism to another and to ultimately have that gene or genes function in the new host environment as they did in the environment of the mother cell. The implications of this potential are staggering and especially so when applied to the enhancement of agricultural productivity.

The protoplast ("single cell") generation of plants and the somatic hybridization of plants utilizing protoplasts represent new research techniques with the capability of providing new biotechnologies for the enhancement of agriculture. Protoplast generation of plants involves the isolation of cells from plants and removal of the cell wall releasing the protoplast from which, under proper culture conditions, it is induced to divide, multiply, and eventually develop into a mature plant. Somatic hybridization involves placing individual protoplasts from different plants, either different individuals within a species or individuals from different genera, and causing the protoplasts to fuse and subsequently develop into a mature plant. A number of intraspecific crosses and a few intergeneric crosses have been made in this fashion. These techniques provide the opportunity for crossing plants that were heretofore reproductively isolated. The protoplast also provides an opportunity, using recombinant DNA techniques, for the introduction of exotic genes into a given plant.

Contemporary molecular and cellular genetics techniques will have profound effects on agriculture. The following are examples of potential effects:

1. Traditional methods of plant improvement will be enhanced by the recombinant DNA and somatic hybridization technologies. These techniques will be particularly useful in developing plants resistant to disease, insects, nematodes, and physical stresses such as cold injury and drought. These efforts will be characterized by identification, chemical description, and cloning of desired genes in the laboratory, and the ultimate insertion of such genes into appropriate plant species. Somatic hybridization will make it possible to bring together pools of genes that have heretofore been isolated as a result of reproductive incompatibilities between species and, in particular, between genera. The "tomato-potato hybrid" is just one example illustrating the potential for the development of a new generation of crop plants.

2. Animal improvement will be enhanced by the application of these new techniques in the areas of animal health, animal nutrition, and reproduction. Hybridoma-monoclonal antibody techniques have been and will continue to be used in the development of vaccines for various animal pests. Opportunities exist for the modification of microorganisms inhabiting the digestive system of the ruminant with the modifications resulting in an enhancement of the ability of microorganisms and the animal to digest forages and feedstuffs. This will be particularly beneficial in the tropical and subtropical regions of the world where difficulties to digest forages abound.

3. Root associations such as the nitrogen-fixation system and mycorrhizae also provide opportunities for the application of these research

techniques for the enhancement of plant productivity. Increases in the efficiency of existing nitrogen-fixation systems, the introduction of nitrogen-fixation genes into non-legume plants and/or the introduction of nitrogen-fixation genes into microorganisms other than Rhizobium and genetic modification of the bacteria involved in associative nitrogen-fixation represent approaches to increasing the overall ability of plants to utilize atmospheric nitrogen. Mycorrhizae are fungus-root associations which occur on the root systems of most plants. They are involved in the uptake of water and certain nutrients and the application of molecular and cellular genetics techniques to this system will result in an increase in the efficiency with which plants can remove water and certain nutrients from the soil.

4. These new techniques also have significant potential for reducing the biological and physical stress on plants and animals. In addition to the capability for modifying the resistance of individual plants to stress, these techniques also provide opportunities for enhancing the biological control of insects, diseases, nematodes, and weeds. Modification of viral, bacterial, and fungal pathogens that attack various pests and manipulation of sexual reproduction in pests are two areas particularly susceptible to development through the use of these new techniques.

5. Food processing, food safety, and food quality will also be favorably impacted by the application of molecular and cellular genetics techniques for the modification of microorganisms involved in processing, food spoilage, and quality reduction in general.

6. The development of alternate sources of energy with emphasis on biomass for methane and alcohol production will also be impacted. There is interest in the development of energy crops and the development of energy crops involves the application of techniques and technologies similar to those which have been used to develop our commercial food, fiber and horticultural crops. The development of energy crops represents a major effort in states such as Florida and the focus is generally on non-food crops. Molecular and cellular genetics techniques are being used in the development of energy crops and in increasing the efficiency of microorganisms in the reduction of biomass to simple carbohydrates and their subsequent conversion to methane or alcohol.

The effective application of contemporary molecular and cellular genetics research techniques to agriculture requires individuals trained in these techniques at both scientist and technician levels. The explosive development of a new set of biotechnology firms, the logarithmic increase in investment in the new biotechnologies by a variety of well established firms and the redirection of programs and program increases at many academic institutions have contributed to the shortage of individuals trained and qualified to do research in the application of these research techniques to agriculture. There is increased competition for qualified senior scientists, scientists at the entry level and for qualified technicians. This shortage of talent in the molecular and cellular genetics areas aggravates an already serious shortfall of talent across many key sectors in the agricultural sciences. This problem has been analyzed in detail by the Office of Higher Education of the Science and Education Administration of the USDA.[1]

In general, this talent shortage is having negative impact on the ability of the United States to maintain its leadership role in the development of innovative technologies and their application not only to agriculture but in other sectors of industry. Among factors which suggest the position of the United States is slipping is the production of Ph.D.'s. During the past year, the United States produced approximately 100 Ph.D.'s in the area of the new biotechnologies while Japan turned out between six and eight hundred.[2] Outside the biotechnology fields there are also indications of the U.S. losing its leadership in technology innovation and application. This is particularly true in engineering.[3] It is imperative that we recognize the shortfall of talent in these areas and the balance of my presentation is based on the assumption that such a shortage exists and that it will persist indefinitely unless something is done.

Graduate education, the training of scientists, occurs in the context of a research program in the setting of an academic institution. Technical training occurs in the same setting but also may be accomplished in special kinds of training efforts and workshops. By virtue of this relationship, any effort to increase the production of scientists will be accompanied by an increase in the overall research effort. This is a simple but important relationship to understand when one begins to consider mechanisms for resolving the current talent shortage.

It is also important to recognize the need for an increase in our research effort in the areas of molecular and cellular genetics if we are to approach attaining the potential benefits outlined above. This increased research effort can be fulfilled in part by the redirection of already existing resources. However, the major emphasis will come from an addition of resources for this purpose since the development of these new biotechnologies for agricultural improvement will involve an integration of classical and contemporary research techniques.

I propose the talent shortage described above can largely be resolved by an enhancement of agricultural research and graduate education programs of the Land-Grant University System and that this solution can be brought about at a minimum cost. Individuals outside the Land-Grant System often fail to recognize the Land-Grant Universities have contributed, and contributed rather elegantly, to basic science. Land-Grant Universities such as the University of

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[1] Coulter, [illegible]. K. and M. Starton. 1980 Graduates of Higher Education in the Food and Agricultural Sciences: An Analysis of Supply/Demand Relationships. Vol. 1, Agriculture, Natural Resources and Veterinary Medicine. Misc. Pub. 1385, 194 p.

[2] Johnson, C. 1982. Growing Pains in the Biotechnology Industry. Conference on the Biotechnology Industry. The Energy Bureau.

[3] Tesar, D. 1982. Testimony on National Engineering and Science Manpower Act of 1982. (H.R. 5254). College of Engineering, Univ. of Fla. 24 p.

California-Davis, the University of Wisconsin, the University of Minnesota, Purdue University, the University of Illinois, Michigan State University, Cornell University, North Carolina State University, Texas A&M University, and the University of Florida have and will continue to contribute significantly to science, to agricultural science in particular, and to the education of scientists.

The Land-Grant Universities were developed as a result of legislation enacted in the mid 1800's. This legislation provided the basis and the mandate for each state to develop such an institution. Approximately 60 percent of the agricultural research in the United States is performed by the Land-Grant Universities.

The Agricultural Experiment Stations of the various states find their origin in similar legislation and the Agricultural Experiment Stations "house" the agricultural research programs of the Land-Grant Universities. Agricultural scientists in Land-Grant Institutions occupy faculty positions in the University and hold appointments in the Agricultural Experiment Station. Support for their research consists of a blend of state, federal formula, and industrial and government grant and contract dollars.

The physical plant of the Agricultural Experiment Stations of the Land-Grant Universities has been valued at a minimum of eight billion dollars. The combined research budget of the Agricultural Experiment Stations is in excess of one billion dollars. This research program involves activities of approximately 14,000 scientists directing a part or all of their efforts to the full spectrum of problems that confront agriculture. By comparison, the research budget of the Agricultural Research Service of the U.S. Department of Agriculture is in the order of 450 million dollars and the agricultural research component in the private sector is valued at 250 million dollars.[4]

It is important to realize that graduate education, i.e., the training of scientists, in agriculture and related areas occurs in the Land-Grant Universities and, in particular, in the agricultural research and education programs of the Land-Grant Universities; there is little, if any, educational activity in the Agricultural Research Service of USDA and in the research programs in the private sector. There is also an educational component related to agricultural sciences in the non-Land-Grant Universities. But the Land-Grant Universities have turned out the majority of the scientists conducting agricultural research in this country today.

Approximately ten percent of the scientists in the Agricultural Experiment Stations of the Land-Grant Institutions are currently involved in molecular and cellular genetics research. The Land-Grant Universities have been and are moving to increase their capability in research and educational programs in these areas. The infrastructure of the Land-Grant Universities provides an ideal setting for the education and development of scientists in agriculture and related sciences. They also possess the technology transfer mechanism, i.e., the Extension Service, which accomplishes the final steps in technology development and utilization.

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[4] Fulkerson, J. F. 1982. Personal Communication. U.S. Department of Agriculture, Cooperative State Research Service.

In addition to possessing the infrastructure and capability for the education of scientists and technicians in molecular and cellular genetics, the Land-Grant Universities also possess the capability and have the structure for coordination of agricultural research at the state, regional, and national levels. Coordination is required in order to minimize duplication of effort and to provide free exchange of information and ideas among scientists. The regional and interregional research project system of the Agricultural Experiment Stations administered through the Cooperative State Research Service of the U.S. Department of Agriculture is the mechanism which provides for this coordination and at the same time possesses the flexibility to provide scientists with maximum opportunity to pursue their own ideas and research interests.

Regional and interregional projects which, by virtue of coordination of the activities of a number of scientists, have resulted in the development of cohesive regional and national research programs. An excellent example is North Central Regional Project 141 entitled "The Chemistry of Wet and Dry Atmospheric Deposition." This project grew out of the concern for acid rain in the late 60's and early 70's. The project was initiated in the mid 70's and at the present time involves the activities of scientists from approximately 30 states. The project is funded by a variety of sources including the Agricultural Experiment Stations, the U.S. Department of Agriculture, the electric power industry and others. This project recently has been proposed for elevation to an interregional project and it is likely this will occur by October 1, 1982. Through the auspices of this project, fundamental to basic research as well as applied research is being utilized to provide a better understanding of the "acid" precipitation problem. This is an excellent example of how the Agricultural Experiment Stations of the Land-Grant University System can combine and focus their research efforts into regional and national programs of national and international consequence. A southern regional project proposal entitled "Molecular and Cellular Genetics for Crop Improvement" is near completion and should be approved by October of 1982. Other regions have initiated similar projects.

In summary, the Land-Grant Universities have the mission to provide research and development for agricultural industries in this country, they have a record of outstanding performance in providing research and development for the agricultural industries, they have the physical plant, the base faculty, support for existing research and development programs, a mechanism for coordinating research at the state, regional, and national levels, the type of infrastructure required in the training and education of scientists in molecular and cellular genetics and related sciences, a history of achievement in graduate education and a technology transfer system in the form of the Extension Service. The system of Land-Grant Universities provides an ideal mechanism for increasing research and development in the areas of the new biotechnologies and for the training and education of scientists in these areas.

This could be accomplished at a minimum of cost to society with a few new resources for physical plant, and emphasis on additional support for graduate students and technicians and for equipment and operating funds to support the scientists, graduate students, and technicians. The Resident Instruction Committee on Policy of the Division of Agriculture of the National Land-Grant Association has proposed a 12.4 million dollar graduate education program that would be administered by the Science and Education Administration of the USDA. Funds would be used as stipends to support graduate education and state institutions would compete for the funds at the national level. This program, if funded, would provide for the enrollment of approximately 670 graduate students during the first year.

Increases in federal formula dollars for agricultural research and in funds available in the competitive grants program, earmarked for research in molecular and cellular genetics would enhance agricultural research programs in the Land-Grant Universities and related programs in the non-Land Grant Institutions. Serious consideration should be given to this alternative.

It is also important to consider industry involvement in supporting these research and educational activities. Industries involved in the development of biotechnologies will only thrive and prosper if appropriate talent is available for them to grow. Industry has supported elements of research and educational programs in the public sector and there are indications of a willingness to increase support levels. It is important that additional attention be given to the development of incentives that would result in increased industry participation in agricultural research and education programs. The Division of Agriculture of the National Association of State Universities and Land Grant Colleges has recognized this need and has appointed a committee entitled the Division of Agriculture Committee on Genetic Engineering to examine alternate sources of funding for agricultural research with emphasis on the development of legislation that would provide additional incentives to industry support. I am the Chairman of that committee and we will be making a report to the Association at the annual meeting this fall. This same committee has also been requested to examine the manpower issue and to develop a set of recommendations to the Association on how this issue might be resolved.

It has been proposed that agricultural research be augmented by the development of a series of national centers and that these centers be located in the various regions of the United States or that they be at one location and developed in pattern after the National Institutes of Health.[5/] While these proposals have merit, enhancement of already existing programs at the Land Grant Universities in the context of the State Agricultural Experiment Stations and the format I have laid out above would not only provide an increase in the research effort in this important area, but also would be conducted in a setting where graduate education exists and thus much of the investment in research would have the added favorable impact of the development of new scientists.

Thus, if the United States is to maintain its leadership in the world in the area of innovative development of high technologies and in particular the biotechnologies and if the United States is to maintain its unequaled or dominant position in world agriculture from the standpoint of research and development as well as overall production efficiency it must increase the rate at which it is training and educating scientists in the knowhow of these new technologies. I propose that this be accomplished through the system of Agricultural Experiment Stations of the Land Grant Universities, the system that has been responsible for the development of the most productive agricultural industry in the world and further that the desired goal can be achieved with this mechanism and at a cost much below that associated with the development of a new series of research centers or a "National Institute of Agriculture." A quantum increase in research and education program support is needed and needed immediately. The mechanism is in place! It is simply a matter of recognition of this fact and taking the appropriate action which will make it possible to capitalize on society's tremendous investment in the system.

[5/] 1982. White House Aims to Shake Up Agricultural R&D. Science & Government Report. Vol. 12, No. 10.

Mr. Gore. Thank you very much. Let me just start off with a question by picking up on your concerns about the manpower. One of the metaphors that is used most frequently in the Congress today is a biological metaphor. The United States is said to be "eating its seed corn," and you seem to be saying precisely that in your discussion of the manpower shortages in this area of plant biology.

Do you believe that this shortage is compounded by the developments in the commercial area and the new relationships between corporations and universities?

Dr. Wood. Yes, to a degree. As I indicated, the tremendous increase in effort in the private sector, the fact that 150 or so new firms have emerged in recent years, this definitely aggravates the situation. In fact, one of the real positive aspects of the institution-industry tie is that it makes it possible for both the university and the industry, when an individual scientist is involved, to have access to the talents of a given scientist. This bridge provides that, and if it is used properly—and I certainly think it can be and is—it is in a sense a strength.

However, there is no question in my mind that the increase in number of new companies and the increase in interest by existing companies and so on, yes, are aggravating the manpower situation.

Mr. Gore. Dr. Brill, let me ask you a variant of the same question. You make the observation in your paper that one of the responsibilities of the Federal Government is to insure a baseline of basic research in this area, and I certainly agree, but let me take a hypothetical case. Suppose a student is trained with Federal support as an undergraduate, goes through graduate school on a grant from the National Cancer Institute, and goes to Madison to work with you and Dr. Hall on a postdoctoral fellowship from USDA. He shows up on campus and is then offered twice as much money to go to work for one of the companies in Madison, and off he goes. What happens to the Government's investment to insure the supply of basic knowledge that you referred to?

Dr. Brill. Well, I really do not see a problem with that.

Mr. Gore. Let me ask you, is that merely a hypothetical or do you know of any such cases?

Dr. Brill. I know of cases of individuals who have gone through my lab, one working with my company and one working with a competing company, both in Madison.

In the case of my company, that individual is not at all following up what he was working on in my laboratory, so he has switched careers. He had opportunities to work in several industries and also several universities and he chose to work in an industry. He is a minority, though. I have around 20 people each year in my laboratory and most of them have taken jobs in universities.

It is not as simple as just a doubling in salary. However, they take a risk, working with both the large companies and the small companies in applied biotechnology. The risk is that the industry may not be around in a couple of years. If they are not around, there may be a glut—and I am predicting something like that—a glut of these individuals on the market because many of these industries will fold, and when they fold there will be people looking for jobs. Then they may go back and take a university job.

Therefore, there is a different kind of lifestyle in an industry versus a university, and most outstanding people tend to join the universities rather than the industries. One of the major problems is that there are very few available jobs in the universities, so even though there is a tremendous interest in plant biotechnology, the jobs in the universities are not opening up to participate in that interest. Universities are in trouble.

Hordes of professors are not leaving universities and going to industry. There are less than a handful at the University of Wisconsin and there will probably remain less than a handful over the next years out of hundreds of professors. As these professors leave, and these professors are generally the more famous ones, the ones who are getting big salaries in the university, then new, young, assistant professors can come in. These people will probably be much more valuable to the university in the long run. The famous ones are busy doing things like testifying before congressional committees.

Mr. GORE. Well, let me follow up by noting that in your prepared statement you say, "A corporation should not be able to buy a department's knowledge." I would tend to agree with you, although many emerging arrangements seem to be focused on doing precisely that.

You are both a corporate figure and a faculty member, and I welcome the invitation in your statement to discuss these issues. The statement, coming from you, seems a little odd. Maybe it should not and I want you to correct my impression. It seems that your corporation would not have to bother paying for the department's knowledge, insofar as you are running both.

Dr. BRILL. Well, you may be touching on two things. One of them is the possibility that the company will take knowledge from my department. It has been arranged that the company is not based out of my research. My university research is very easy to describe and work going on in the company really bears hardly any relation to what is going on in the university, unlike some companies where a professor has made a discovery and arranges a company around his discovery or around his interests. Therefore, bridging that gap has not yet been a problem.

The other thing, and in fact I think that relates really more to your first question, and that is that when chemists go out to the many jobs in the chemistry industry, nobody asks, "Is that fair?" The Federal Government has paid for a fair percentage of their training and they are working for industry rather than going into a university. I think it is the same thing here. I do not think there is anything special about this.

Mr. GORE. Well, the fairness question in that specific context is secondary to the need to sustain an adequate level of training to maintain this research base, and Dr. Wood has expressed serious concerns about the shortage of teachers, of postdocs willing to fulfill this mission.

Dr. Wood, isn't it your testimony that we have a very troubling shortage of these people?

Dr. WOOD. Yes. I would like to comment on a few of the points made by Dr. Brill because I agree with most everything he said except, I think, one issue. Of these 150 or so firms that have evolved or emerged in the last few years, it has been forecast that about 50 or so would be going out of business, if they have not already done so, so there is no question that life is different in the business community. It was one of the factors I was thinking of when I said there are factors other than salary considerations. One is the kind of a situation a scientist finds him or herself in, in the academic environment in contrast to the industrial environment.

Mr. GORE. Of course, there are beginning to be some risks on campus, too.

Dr. WOOD. That is right. That is exactly right.

Mr. GORE. Go ahead.

Dr. WOOD. I think while we do not see in the immediate future a lot of new positions at the universities, unless additional funding sources become available, there is a general wave of redirection occurring. As I talk to experiment station directors, deans of land-grant colleges, as I talk to individuals in the Division of Agriculture of the Association and study these kinds of problems, right now we have a problem and in the long haul we see a serious shortage and I see the problem becoming even worse if something is not done now.

If additional resources are not made available, then this will in turn aggravate the situation. Now as I said during the verbal comments I made earlier, we do not have a number; that is, we have not quantified the need. We are trying to get at exactly what the need might be, but institutions that have attempted to, say, just double the number of scientists they have in this area, as we have in the last 2 to 3 years, have had difficulties.

We have not been raided. I do not want to give that impression. We have lost two or three scientists to industry. We have not competed favorably in a couple of search and screening situations but it is becoming more and more difficult, and we are looking at a dozen or so positions in Florida right now, new positions in fact that we got from the State this year, and some of the chairmen of our respective departments that these will end up in are voicing concern about being able to find anyone that would really have the kind of talents we need.

Mr. GORE. Dr. Brill, do you want to respond?

Dr. BRILL. My experience is certainly much narrower than Dr. Wood's but in my university department, I guess the last three or so people that have been hired are molecular biology type people and we really did not have any trouble finding very good people. That is my only experience there.

Mr. GORE. Well, that may be because you are in the department, doctor. Modesty may prevent you from commenting on that——

Dr. BRILL. No, no, no. That has nothing to do with it.

Mr. GORE. But the fact is that some departments have an easier time attracting—I mean, students gravitate toward leading thinkers in the field. I think it is the same with companies. The conversations I have had with corporate figures in this area indicate that the strongest and most vital of the corporations in this area are not having difficulty in getting the talent they need but the ones that are one tier below are starving to death.

Dr. BRILL. Well, it is clear that if more Federal funds or State funds were available to universities, that more individuals would be attracted to these positions. The problem is that there are not that many openings, period. Florida may be an exception but most universities are really clamping down now in all of these areas. There was a flurry of interest in biotechnology and I think the ones who got in early are set, and so there may be specific problems right now with people or institutions that are trying to get involved in biotechnology 5 years later than some of the other universities.

However, industry is really taking up the slack that the universities and the Federal Government are not fulfilling, by providing positions for these personnel. In fact, one of the reasons that biotechnology with respect to plants is as primitive as it is compared to the other areas of research is that if one wanted to get a good job working with plants, there were very few available. There were no molecular biology institutions focusing on plants. There were no equivalents of cancer institutes around. There were no NIH special grant equivalents.

Mr. **Gore**. No.

Dr. **Brill**. Now that people and their students can see positions opening up, now there is incentive for more people to get into university positions as well. There is money available, there is a future in it, whereas there was not before.

Mr. **Gore**. Let me state an impression I have and ask you, the witnesses, to correct it insofar as it needs correction.

First, there is a general impression in the country that genetic engineering breakthroughs with commercial applications will appear first in plant biology. That impression in the country may well be wrong because the earlier breakthroughs in chemistry and the dramatic results of the breeding program gave plant biology a vigorous kick that improved productivity so dramatically that there was relatively less attention paid to biotechnology or cellular biology in the plant area than there was in animals and mammals. As a result, we now see a much more sophisticated body of knowledge about the cellular biology and DNA structure of animals than we do with respect to plants and there is now, as the productivity upsurge that accompanied this last body of breakthroughs in plants is now running its course and productivity increases are slowing down, there is a scramble to find people who are trained in cellular biology of plants and a shocking lack of understanding of some of the basics of plant biology that have already been found in animal biology.

Comment on that impression, if you would. Is that right or wrong or inaccurate? Who wants to go first? Dr. Brill?

Dr. **Brill**. The first statement that you made is correct. The statement that the first commercial uses of biotechnology will be in plants is incorrect.

Mr. **Gore**. I said that was an impression out in the country.

Dr. **Brill**. Oh, I see.

Mr. **Gore**. I said that that impression is probably incorrect.

Dr. **Brill**. Right. That is definitely incorrect. There are a lot of vaccines that are coming out. There are diagnostics that are coming out. There is the production of insulin, the production of interferon, growth hormones and things like that. Success has been achieved. The next step is just scaling up and marketing and selling——

Mr. GORE. However, where plants are concerned there is a big hurdle that we still have to get over, and in some respects it is a hurdle that the animal biologists have already gone over.

Dr. BRILL. That is right. We are making up for the complacency of the United States or of people in general with respect to plants. The reason people are interested in animals is because of diseases, and I believe that the incentive for putting money previously into animal problems is for our own personal, selfish health interests, whereas none of us is starving or really know friends who are starving. We all have been sick and know people who have been sick. I do not know if that is the reason for it but there certainly has not been any groundswell of interest in plants whatsoever until very, very recently.

Mr. GORE. However, now that it is occurring we are finding that the gaps in knowledge are enormous.

Dr. BRILL. Absolutely.

Mr. GORE. As a result, is it fair to say that the predictions in the area of plant biology for dramatic breakthroughs very soon may have been hyped a little bit?

Dr. BRILL. Yes, definitely. The press has hyped almost everything. There are some short-term breakthroughs that will be applied by a variety of companies in the next 5 years. The long-term ones, genetically engineering plants, plants that will be used out in the field by the farmer; that takes many, many breakthroughs.

Again, nobody has genetically engineered any plant yet to do anything of interest so it could take quite a while. However, I am just tempering that by saying that surprises are coming rapidly.

Mr. GORE. Yes.

Dr. BRILL. However, certainly we lag in our basic knowledge far behind the equivalent areas in animals.

Mr. GORE. In animal biology.

Dr. Mott, could you comment on the earlier general statement I made?

Dr. MOTT. Yes. I would agree with it, with a couple of additions. One is, there is a backlog. You are correct in the lack of the real basic science undergirding to support a big thrust in that area, which already exists in the animal situation for reasons we have discussed.

However, there is also a bit of a backlog of expertise and technology which is not being used yet by the agricultural system. Those will probably be the basis of some early breakthroughs. For instance, we should take advantage of much bench science already existing in the lab in terms of making genetic crosses more successfully or easier. There is a certain tradition that goes along with what methods have been followed, and standard breeding programs are productive but there has been a slowness to pick up on the new technology as it comes out. There is a backlog of just practical technology that I would imagine could be used. That may be the basis of some of the earlier breakthroughs that come in the plants.

However, I would expect those breakthroughs to be followed by a drought because the well is dry. You run out of the basic undergirding which is necessary, and that brings me to the second point.

There is a recognized need for that undergirding now but some problem comes with directing attention to it in semantics. You get attention on campuses and in industry for "biotechnology" and then you begin to realize the obstacles to applied use. We need to know more about the enzymes, more about the metabolism, more about the physiology of plants and how they do their thing. The common reply is, "That is not biotechnology, that is just basic science,"and it does not fit under the nice umbrella of the term "biotechnology." Indeed, I would agree that it doesn't sound like biotechnology, and that is one of the problems we have. We need to do some hard work, some basic hard work which is hard to relate to a breakthrough tomorrow. This work has been done over the last 20 or 30 years in medical fields and in other areas under a number of different guises but it has been done, it has been happening. We need the knowledge for plants.

Therefore, now as we are pressed to catch up on some of this hard work, it just does not ring quite the same bells if you talk about basic science as it does when you can twist it around to look like "biotechnology."

Mr. GORE. It does not have the same sex appeal.

Dr. MOTT. Yes, right.

Mr. GORE. Dr. Wood?

Dr. WOOD. I would like to make a comment. I basically agree. I think you said it very well. I would add, though, that in the area of plants—and I would appreciate these gentlemen's comments on this—in the area of plants probably the next surge of improvement is going to come from the area that Dr. Mott talked about this morning, the protoplast generation and the somatic hybridization of plants, rather than in fact the recombinant DNA and the application of recombinant DNA technology to——

Mr. GORE. Put that into plain language, will you?

Dr. WOOD. Yes, sir. It has been demonstrated that we can take a protoplast from one plant and a protoplast from another—essentially a single cell—bring a single cell from each of two different plants together in a culture in the laboratory, have them fuse and have an adult plant develop. It has been done with tobacco, carrots, petunias, and so on.

Interestingly, they have been able to fuse protoplasts of tomato and potato and get a hybrid. These plants are normally reproductively isolated. They do not freely reproduce together sexually in the field.

Therefore, the extension of this kind of technology, the whole area of tissue culture, protoplast culture, and the somatic hybridization, or the ability to cross plants outside the sexual reproductive process that normally occurs in nature, opens up many opportunities for bringing gene pools together that heretofore just have not gotten together in nature. I really think that with individuals trained and educated in the area of tissue culture and protoplast culture, working hand in hand with plant breeders will bring different kinds of genes together, bypassing sexual reproduction, and improving plants and crops.

I really think there will be a tremendous surge in the improvement of plants using this technique in the next 5 to 10 years, and it may precede the surge of the true application of the molecular biology of recombinant DNA.

Mr. GORE. Well, now, if we are in this period where molecular plant biology—what is the best descriptive label for this area?

Dr. BRILL. Plant biotechnology.

Mr. GORE. Plant biotechnology. OK. Agrogenetic engineering? You do not like that one. All right, plant biotechnology.

If we are in this area where plant biotechnology is newly confronting the lack of basic knowledge impeding the prospective breakthroughs, and there is a scramble for more scientists and researchers to come into this area, and 150 to 200, give or take 50 companies are scrambling to get all of the new thinkers coming off the assembly line that they can, how then are we going to address this question in the universities? How are we going to make up for this shortage of talent in the universities?

Dr. MOTT. I do not see—again, I would agree with Dr. Brill in the sense that, you can define areas which need investigators, need trained people, professors in that area. In these times of shrinking university budgets it is a bit difficult to create such faculty positions. I do not see the difficulty being so much that trained people are drained off into industry but rather, student enrollment is decreasing in the universities; we do not have the faculty positions there begging to be filled. "Shall we hire a classical geneticist or a new molecular biologist?" We do not have many of those decisions coming around.

Mr. GORE. Because the students are just not getting into those areas, is that what you are saying?

Dr. MOTT. Well, the students are there; it is new faculty positions that are lacking. The easy way would be to build a layer upon what we have, a new layer dealing with plant biotechnology, if you will. Create and staff a new area of molecular biology of plants, and not diminish the applied programs and the very productive programs we have had in the past which have brought about the kind of agriculture we experience in this country.

When one is now faced with limited budgets and so forth, saying, "Let's take away some of those and change them to a new label," that is a very difficult thing and it is not happening all that fast. We do not have that many positions at our own university to deal with.

Mr. GORE. The point I am getting at—let me reframe the question—we are losing 3 million acres a year in the United States to erosion and blacktop. We are seeing an increased demand for America's agricultural products in the rest of the world because of population increases, because the price of fertilizer has made it more difficult for other countries to feed as large a percentage of their people.

If we are to protect our resources, both in terms of land and the impending water shortages in agricultural areas in the West, and at the same time meet that portion of America's destiny which includes feeding more of the world's people, it is obvious to me that in the balance of this century and on into the next we ought to stimulate developments in this area of plant biotechnology. In order to do so, we ought to be training more qualified people in this field.

Now let me ask you first of all if you have any reason for disagreeing with that or disagreeing with the sense of urgency which I attach to that and, secondly, how do we do it? Dr. Brill?

Dr. BRILL. Well, we are now somewhat addressing that urgency. One is the new USDA competitive grants program, allowing people, not necessarily in agriculture schools, to apply for money to do creative work in the basic plant sciences. That money has allowed laboratories, laboratories that usually handle one graduate student, maybe two, to now handle a postdoc, so there have been over the past few years an increase of talented young people, students and postdocs, going into the plant sciences where that did not exist before.

Therefore, there is a mechanism and the number of good students is certainly increasing. It is not increasing at a rapid enough rate. I also want to stress that it is not numbers that is important, it is quality, and that is where I would recommend that you place most stress for the future.

There is another point, that many or probably most of the people who have made the breakthroughs in genetic engineering of plants are not trained as plant researches. They are mainly trained as bacteriologists or virologists, and there are people, very good also coming in from a variety of disciplies, including the animal sciences, into plant sciences. Again, I want to stress that quality is much, much more important than quantity.

Mr. GORE. Let me recognize my colleague, Mr. Volkmer, not just for the 5 minutes but we will come back for additional questions.

Mr. VOLKMER. I will take my 5 minutes and then I have a rollcall. We have a vote.

Mr. GORE. Yes.

Mr. VOLKMER. I would just continue on with the questioning of the chairman and listening to your answers. I am sorry I was not able to be here before but I only have four committee meetings this morning, going on at the same time.

My impression from exactly the last statement and others, the answers before, is that perhaps additional funding is needed to be made available to universities in the research area if we are going to have an increase in the number of graduates or graduate students in the area specifically of what you call plant biotechnology. Do you agree on that or disagree?

Dr. WOOD. I did not get that as a question. I am sorry.

Mr. VOLKMER. The question is, is it a question of funds to enable us to have additional graduate students in the area of study of plant biotechnology?

Dr. WOOD. Is there a need for additional funds?

Mr. VOLKMER. Yes.

Dr. WOOD. I would say "yes."

Mr. VOLKMER. Even if we do not have the additional funds and you continue to operate the way you are, will we have sufficient personnel, do you feel, within the next 5 to 10 years both for university study and research and also for that which it is envisioned will be necessary in private business?

Dr. WOOD. From where I view the problem, no.

Mr. VOLKMER. All right.

Dr. **Wood**. There will be redirection. There is currently redirection going on. As Dr. Mott has indicated, it is a slow process. It varies from one institution to another. Some of the slack will be taken up by redirection of existing programs but in the agricultural sciences there are other activities that we are required to do to continue to provide the technology support, that is, the research support for industry in the country. Therefore, there is only a certain amount of redirection that can be done with existing resources. In my opinion and the opinions of others, we do need additional resources.

Mr. **Volkmer**. Besides the U.S. Government, whether it is from the USDA or NSF or any other place, do you envision increased resources, let's say, Dr. Wood, from your own university?

Dr. **Wood**. Yes. We see increases coming from not only the Federal and the State but also the private sector. An area that is receiving a lot of attention now on the part of some people involved in college administration is the development of legislation that would provide additional incentives for industry participation. There is a tremendous opportunity here. There is going to have to be a general base of support derived from those three sectors, the Federal, the State, and the private.

Mr. **Volkmer**. You welcome industry participation in university research?

Dr. **Wood**. Yes, we always have and I think we always will. There are mechanisms whereby industries and institutions can cooperate in this area and the rights of all can be protected.

Mr. **Volkmer**. Dr. Brill and Dr. Mott, do you have any comments on any of those statements of Dr. Wood?

Dr. **Mott**. I see the industrial input of funds as being a positive thing which has allowed, in my own experience and others I am familiar with, allowed a bridging of disciplines and an assembly of a long-term effort which can be done more quickly and more firmly along that avenue than it can be done within the processes of the university if you are dealing with State moneys, for instance.

Therefore, in that sense it has allowed a bridge between schools and between departments to bring together the needed expertise to make some of these marriages of the biotechnology that we have been interested in. Those kinds of arrangements can vary from entirely public domain commitments to something having to do with some proprietary interests. But it seems that industrial money does not necessarily impose any crimping of style in terms of the science or the university. Therefore, I view it as a positive thing.

Mr. **Volkmer**. Dr. Brill?

Dr. **Brill**. Well, there is definitely a need for money to get our basic knowledge of plants increased, not so much money to get that knowledge applied. Industry will play a major role there. Industry is stepping in and participating in research at universities.

Mr. **Volkmer**. Thank you, Mr. Chairman.

Mr. **Gore**. Thank you very much.

I have other questions that I would like to ask this panel. I would normally go to the next panel during a break but with your indulgence and the indulgence of the other witnesses, we have a vote on the floor on the conservation corps, to provide federally funded jobs for many of the people that we failed to give the talents in this area that we should have when we could have trained them, so we will adjourn for 6 minutes and then come back.

[Recess taken.]

Mr. GORE. The subcommittee will come back to order.

I certainly appreciate the patience of our witnesses and the guests of the subcommittee.

If we are going to do a better job of training people in this area that is so important, obviously the Federal Government and the land grant-university system in particular is going to have to play a significant role in this regard. I want to quote from your statement, Dr. Wood. You say,

> I propose the talent shortage described above can largely be resolved by an enhancement of agricultural research and graduate education programs of the land grant university system, and that this solution can be brought about at a minimum cost.

First of all with respect to the Agricultural Research Service, would you three witnesses agree that there has been a misdirection of resources? Let me rephrase that. Has there been too small an allocation of resources with regard to this particular area?

Dr. WOOD. To the Agricultural Research Service?

Mr. GORE. Within the Agricultural Research Service to the plant biotechnology area. We have a witness later who will have equal time, and we will try to triangulate on the truth here.

Dr. WOOD. I was going to say if you are asking the question, has there been enough direction of effort within the Agricultural Research Service of USDA in this area, I would have to defer to Dr. Gerald Still.

Mr. GORE. Well, what is your opinion?

Dr. WOOD. I do not know how much they are directing to this area.

Mr. GORE. Oh, OK. All right.

Well, now what is your opinion of the White House Office of Science and Technology Policy calling this meeting to investigate their opinion that,

> The land grant system has increasingly focused on short-term, applied problems of direct concern to State farming communities, to the neglect of scientific developments that have proven revolutionary in other areas of the life sciences.

Would you agree with that analysis with respect to the land-grant system?

Dr. WOOD. No, I would not. In fact, I disagree with most of the statements in that particular piece. If I can make a couple of comments to explain, first of all there is a sum cited in there of $900 million that the Federal Government is placing in the land-grant systems for agricultural research in the ag experiment stations. That is not true. The total research budget of the agricultural experiment stations as I indicated earlier, is about $1 billion, and of that a maximum about $200 to $235 million is Federal. The balance of it comes from States, so the $900 million item is way off.

The notion of establishing the National Institutes of Agriculture, which is proposed in that same article, has some merit but I personally think that the same advantages or beneficial effects can be derived by increasing support for agricultural research in the Agricultural Research Service and in the land-grant system, and providing opportunities such as the competitive grants program does for non-land grants to become more involved in agricultural research.

Mr. GORE. OK. Without objection, since you have referred to this article, let me put it into the record at this point. It is the Science and Government Report, the issue of June 1.

[Material to be supplied follows:]

SCIENCE & GOVERNMENT REPORT

12th Year of Publication

The Independent Bulletin of Science Policy

Volume XII, No. 10 P.O. Box 6226A, Washington, D.C. 20015 June 1, 1982

White House Aims to Shake up Agricultural R&D

The White House Science office has teamed up with the Rockefeller Foundation to sponsor a forthcoming strategy session aimed at shaking up the nation's pork-barrel dominated network of agricultural research institutions.

About 10 to 12 ag schools in the nationwide land-grant system are in tune with modern science, Denis J. Prager, Assistant Director of the Office of Science and Technology Policy (OSTP), told the White House Science Council May 20 in a briefing that he gave on the coming meeting. The outsiders, he suggested, are isolated from the mainstream science departments in their institutions. In the meantime, he said, the land-grant system has increasingly focused on short-term, applied problems of direct concern to state farming communities, to the neglect of scientific developments that have proven revolutionary in other areas of the life sciences.

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China Receiving Huge Loan For Science Education—Page 7

The meeting, billed as a "small, highly focused workshop intended to develop a strategy for strengthening America's agricultural research," will be held June 14-15 at the Winrock Conference Center, Morrilton, Arkansas under the title "Workshop on Critical Issues in Agricultural Research." Participation has been limited to 14 persons, drawn from academe, government, high-tech industry, and think tanks.

The theme of scientific backwardness in an agricultural-research enterprise that now absorbs nearly $900 million a year in federal funds has been repeatedly voiced over the past decade by scientific critics outside ag's comfortably insulated system of formula grants. Berating that system from the perspective of peer-reviewed competition for federal research funds, these critics, mainly in the non-ag sectors of academe, got a small competitive grants program going, but otherwise aroused little political backing for their revisionist views.

The difference now is that the Reagan-induced upheaval in federal science policy nicely dovetails with the old criticisms of intellectual sluggishness in ag research, protected enclaves, and an absence of clear boundaries between private and public responsibilities. What isn't different, however, is Capitol Hill's backing for the entrenched system, which has a well-organized nationwide constituency that likes things just as they are.

"A look at the really exciting biological research in this country that is applicable to agriculture," Prager told the Council, shows that "a relatively small percentage of it goes on in the land-grant system."

Offering what he emphasized is "a personal opinion," Prager said that the "land-grant system is in a mode of self-destruction in that it has not adapted well to" outside institutions in the forefront of the biological sciences. "In trying to protect itself," he continued, "the land-grant system is losing some credibility and losing support and becoming more and more isolated." As competition has increased for federal research funds, the land-grant schools, "instead of trying to find a way" to partake in advanced research, have "tended to circle the wagons and try to keep the other people out. So, there is this problem of the adaptation of this tradi-

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In Brief

"The aim is to correct the problems, not get rid of labs," David Packard, head of a White House Science Council panel on the future of the national labs, told a Council meeting last week. Packard, Chairman of the Board of Hewlett-Packard, expressed distaste for layoffs at the labs. "You're not going to have a very reliable system," he said, "if you come to the end of the line and fire 1000 people." Other jobs or transitional arrangements should be provided in cases where programs or whole labs are eliminated, Packard said.

The Department of Agriculture has dropped loyalty checks for members of its peer-review panels after the general press picked up reports of the practice from Science *magazine. What remains to be seen now is whether the Veterans Administration, which has so far escaped the spotlight, goes through with arrangements for setting up its own system of loyalty checks on peer reviewers.*

One of the biggest new packages of money for academe—the Defense Department's $30-million special fund for laboratory equipment—faces an uncertain future as the once-sacrosanct Defense budget goes into the deficit-cutting mill. The money, originally scheduled to start flowing next October 1, was the only federal equipment fund specifically earmarked for university labs.

...Most Ag Scientists "Isolated on Campus"

(Continued from page 1)

tional system to the new biology and the new ways that research is done in this country." Prager, a PhD physiologist, has been a member of the White House science office since 1978.

Referring to the small program of agricultural competitive grants that was started during the Carter Administration, Prager pointed out that it represented a break with agriculture's tradition of formula and block grants—and that year after year, it has encountered heavy political opposition. "It has struggled and struggled throughout its existence not to be wiped out, essentially by the traditional forces in agriculture who are concerned that these funds that go out on a competition can go to any institution, whether it be inside the land-grant system or outside."

The Arkansas meeting, he said, "is to discuss these kinds of issues. Essentially, we will discuss the institutional issues facing agricultural research, and we will attempt to develop a strategy for dealing with those issues; in other words, coming out with an action plan, if we can, of things which the federal government ought to do through our office and the Department of Agriculture, and what the land-grant system ought to do."

Prager said that OSTP Director George A. Keyworth, who was not present during the briefing, had expressed interest in the possibility of having the White House Science Council take an active interest in the agricultural research issues.

The Council drifted off into idle discussion after Prager said he would report on the Arkansas strategy conference at the Council's next bi-monthly meeting. Nonetheless, it was a revealing discussion, opening with Council Chairman Solomon J. Buchsbaum—Executive Vice President of Bell Labs—wisecracking to his colleagues, "Not many farmers around this table."

Not all the ag schools are scientifically backward, Prager said. "A number of these schools are really excellent," he said—among them the University of Wisconsin, the University of California, and Cornell. "In many of those schools," he continued, "the agriculture departments and the other departments are well-integrated."

Conference Participants

The following are listed as participants in the Workshop on Critical Issues in Agricultural Research:

Perry Adkisson, Deputy Chancellor for Agriculture, Texas A&M University System
James T. Bonnen, Professor of Economics, Michigan State University
Winslow R. Briggs, Director, Department of Plant Biology, Carnegie Institution of Washington
Rep. George E. Brown, Jr. (D-Calif.), House Agriculture Committee
Emery Castle, President, Resources for the Future
Irwin Feller, Director, Institute for Policy Research and Evaluation, University Park, Pennsylvania
Ralph Hardy, Central Research and Development, E.I. Dupont
James B. Kendrick, Jr., Vice President, Agriculture & University Services, University of California
Lowell Lewis, Assistant Vice President, Agriculture & University Services, University of California
James Martin, President, University of Arkansas
John Marvel, General Manager, Research Division, Monsanto Agricultural Products Company
John A. Pino, Director for Agricultural Sciences, Rockefeller Foundation
Denis J. Prager, Assistant Director, Office of Science & Technology Policy
Peter van Schaik, Associate Area Director, U.S. Department of Agriculture, Agriculture Research Service

"What do you mean?" a Council member asked.

"They're on the same campus and they share faculty and they teach some courses together," Prager replied. "And in those cases, the formula funds that come from government are a tiny percentage of what they get, because those are sophisticated schools; they get competitive funding from NIH and NSF and DOE...On the other hand there are a lot of other land-grant schools which are not nearly as good and in which the agricultural science is very isolated, and in which that kind of intercourse just does not happen," he added.

"There is a tendency, on the campus as well as nationally, for the agricultural scientists to be isolated,"

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...Land-Grant Community "A Closed Society"

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Prager said. They have their own source of funding in the Department of Agriculture, and they have their own journals, and they go to their own meetings. The land-grant community is a very closed community. They have their own association, the National Association of State Universities and Land-Grant Colleges (NASULGC), which is politically very powerful. And it really in some ways is a closed society.

"If they decide," Prager continued, "that you are not on their side, that you are raising questions about the way this land-grant community operates, they will lock you out of that community. . ."

Asked to describe the status of the Department of Agriculture's competitive grants program, Prager replied that the Reagan Administration was encountering the same opposition that the Carter Administration had experienced. Both regularly sought to increase funds for the program, "and each year, the House Appropriations Committee—[Rep.] Jamie Whitten [D-Miss., Chairman of the Appropriations Committee]—has 'zeroed' it. And it's only through the Senate that we've been able to get a compromise, which is essentially keeping it a flat budget each year. So, we've still got this meager $15 million which we're trying to focus to do high-quality basic science."

A Council member asked whether NASULGC supported the competitive budget.

"Officially," Prager replied, "NASULGC and individuals support it. But, through the back door, there is a consortium, a coalition, particularly of Southern land-grant deans and directors who oppose it and who have gotten to Whitten and Whitten has opposed it."—DSG

An Invitation

Written on White House stationery, the letters of invitation to the Workshop on Critical Issues in Agricultural Research describe the meeting "as the first step in a process designed to critically analyze this country's agricultural research enterprise and identify actions designed to strengthen that enterprise."

Signed by Denis J. Prager, with the title of Assistant Director, Office of the Science Advisor to the President, the letter paints a gloomy picture of the condition of agricultural research and states that the "need for... strengthening seems clear."

National dependence on agricultural productivity "creates the pressure of expectations for America's agricultural research enterprise to sustain the phenomenal level of success it has achieved over the past 100 years," the letter states.

"Yet," it continues, "there is considerable evidence

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Background Paper Discusses NIH Model for Agriculture

A paper by James T. Bonnen, Professor of Economics, Michigan State University, provides an authoritative backdrop for the forthcoming Workshop on Critical Issues in Agricultural Research. Titled "Agriculture's System of Developmental Institutions: Reflections on the US Experience," the paper was delivered last October at an agriculture symposium at the University of Laval, Quebec. Following are excerpts:

●

The nearly complete monopoly of both biological research and the communication of new biological knowledge and technologies to farmers, which the [land-grant] Colleges and the US Department of Agriculture once had, is gone. Such innovations as hybrid corn, other new plant hybrids, high-protein animal feeds, high-analysis fertilizers and the inputs of the chemical revolution are predominantly private-sector activities. . .The genetic engineering of the characteristics of plants is already so attractive to proprietary firms that they are now raiding US university faculties. . .Today the overwhelming majority of dollars spent on agricultural research are expended by private firms...

This raises serious questions about the future roles of the public and private sectors as sources of new knowledge in agriculture. [It has been suggested] that if public research investments are to earn a high rate of return, they must be moved more toward basic research. This is no doubt true, but the Colleges will still have to be concerned to maintain the applied research necessary to protect their state's competitive advantage in agriculture and they must keep a general presence in applied research or expect to see the extension service slowly die for lack of support for its mission. . .

Sadly, for all its past accomplishments. . .USDA [is] now unavoidably dominated by clientele with narrow economic interests. It is likely that national agricultural research must be free of that environment before it will be allowed to perform its function properly. One model that comes to mind is that of the National Institutes of Health. A National Institute of Agriculture probably should be independent of the USDA but responsible to the agriculture committees of Congress. . .

US Cuts off Science Exchange with Poland

The chill between Poland and the US has claimed scientific cooperation as its victim. Amid charges by the Polish news media that American scientists in Warsaw are engaged in spying, and after a raid by Polish police on a meeting of Polish scientists and American diplomats, the State Department has decided to end the bilateral program that the two countries commenced in 1974.

The precipitating event took place on the evening of May 9 in Warsaw, when two American diplomats—John Zerolis, Scientific Attache, and J. Daniel Howard, Cultural Affairs Officer—met with two Polish scientists, Ryszard Herczynski and Wladyslaw Fiszdon, at Herczynski's apartment. The purpose of the meeting, according to the State Department, was to discuss a visit to Warsaw by an official of the National Science Foundation in connection with a research project sponsored by the two governments under the exchange program.

According to the State Department, Zerolis and Howard reported that as they were about to leave, "A number of individuals burst into the apartment, showing no identification whatsoever," and then "manhandled them, and spreadeagled them against the wall and searched them."

When one of the Americans produced an identification card, the intruders "threw his ID across the room." A photographer took about 50 snapshots. A member of the group, presumed by the State Department to be police officers, also confiscated three Solidarity bulletins (underground publications of the outlawed union) and two copies of proposals for scientific exchange grants.

The American ambassador, Francis J. Meehan, protested the raid the following day to the Polish government, but the State Department says no explanation was given. However, on May 12, the US Foreign Broadcast Information Service, which monitors foreign media, picked up a report from the Polish press accusing visiting American scientists of commiting espionage in Poland. Though half the visiting scientists have come to establish legitimate relationships with Polish colleagues, said the report, "it is not hard to note that for many persons...this has been a mere pretext." Certain visitors, it continued, had "totally different interests and had made contacts with "activists on the Solidarity payroll." In the meantime, Zerolis and Howard were given until May 14 to leave the country.

The State Department directly retaliated, on May 13, by ordering the expulsion of the Polish Attache for Science and Technology, Andrzej Koroscik, and a political officer, Mariusz Wozniak. They were given until midnight, May 17, to leave Washington. And the scientific exchange agreement, hobbled since martial law was declared but still alive, was suspended indefinitely.

The exchange program began in the early 1970s, allowing scientists from American universities and Polish institutions to travel back and forth, at a rate of about 100 from each country every year. In 1974, Poland began matching US expenditures in a joint fund, consisting entirely of zlotys, that over the past 10 years has contained a total of about $40 million, according to a State Department source. The exchange covers the gamut of disciplines, but has concentrated especially on agriculture, public health, and energy topics.

Arrangements are typically made between US academics and their Polish colleagues, with eight US federal agencies sponsoring travel in the US, and institutions like the Polish Academy of Sciences taking care of visits to Poland.

The two Polish scientists in the Warsaw raid have been collaborating for 11 years with a Stanford Professor of Chemical Engineering, Andreas Acrivos, but their official relationship began only in 1980. For several years prior to that, the Polish government refus-

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INVITATION *(Continued from page 3)*

that this enterprise—at least the federal-state partnership, traditionally thought of as comprising the enterprise—is in failing health, not able to sustain its level of past performance and not up to meeting our high expectations of it." The letter continues:

"If American agriculture is to survive and flourish as it must if America is to survive and flourish, we have to be assured of the continued development of new knowledge and technologies through agricultural research. Assuring future scientific and technological advances for agriculture will require that we mobilize all appropriate resources including those of the Federal Government, state land-grant colleges, other state and private colleges and universities, non-profit research institutes, and the business and industrial sectors.

"Accordingly, we are inviting a select group of individuals from government, universities, and the private sector to participate in two days of concentrated, unstructured discussions focused on: (1) assessing the degree to which the agricultural research enterprise is up to meeting the challenges it faces; (2) developing a strategy for strengthening that enterprise and mobilizing the public and private sector energy and resources required to assure its future success; and (3) defining specific next steps in implementing the strategy. Although we recognize the integral roles of teaching and extension, the workshop will focus primarily on research."

Mr. GORE. Either of the other witnesses? Dr. Mott?

Dr. MOTT. I would like to make one comment. I do not know all of those figures and the values that are assigned to basic research or physiology of plants or whatever but I would like to make one word of caution. People have not intentionally, I do not think, in the ARS or the USDA or in the country as a whole, intentionally said "Let's get together and neglect some part of plant biology."

Rather, for a long time they did the things that were leading into the kinds of disciplines which bring about crop improvement and increased yield. We are in a new ball game here now, with capabilities of dealing with genes, capabilities of dealing with cells which did not exist or did not exist with the urgency that we can see now. That has called upon a body of knowledge which was not really necessary for the progress we had before and it is very much necessary now. I think there is a tendency to go back and attempt to place blame back there someplace. Maybe one ought to be a little bit cautious with that.

Mr. GORE. Yes. That is a much better way of framing the problem, if one exists, as many people believe it does.

Dr. Brill, you wanted to comment on that.

Dr. BRILL. The ARS has stressed both some basic sciences and the applied sciences; but even in the basic sciences, in the competitive grants program, there are about five areas including photosynthesis and nitrogen fixation. That is much too narrow a focus. We really need basic plant science research without any specific definition, so that we become excellent plant biochemists and molecular biologists.

It is very difficult to predict what talents, what knowledge we are going to need 5 years from now for our applications, and I think it is a little bit dangerous to say we should put our money into certain defined areas.

Mr. GORE. Well, the majority of advances leading to the current level of expertise in biotechnology have come from research supported by the National Institutes of Health and the National Science Foundation. You would agree with that. The heart of this support has been the competitive individual grant system as distinguished from the institutional support programs which have been the main method of support used by USDA.

I know that there is a controversy about the competitive grant system. There may be some disagreement on this panel. Is USDA making an effort to increase the support provided to its own relatively new competitive research grant program, and if so, is that a good idea or a bad idea? Dr. Mott?

Dr. MOTT. I think it is a good idea but the extent to which it is being done I find myself is a little bit lacking.

Mr. GORE. What is your opinion of the competitive grant system in general?

Dr. MOTT. To me as an individual it seems a good thing but it is just not big enough, not big enough to make that much of an impact.

Mr. GORE. Dr. Brill?

Dr. BRILL. It has been very good. It has been a major contributor to some of the interest in plants in the United States, in that very good scientists can compete for money in several areas. The main problem is that it is too narrow, that it should just be funding good, basic plant biology and leave it at that.

Mr. **Gore**. Dr. Wood?

Dr. **Wood**. Yes. To answer your first question on this, there is an effort and there has been efforts to increase this type of funding. We have been relatively unsuccessful in increasing it very much in the last 3 or 4 years. I think the proposed increase for the coming year, if it remains, is about $6 million. It is at $16 million at the present time.

I think there is definitely a need for the competitive grants program, that it brings a dimension to agricultural research in the land grants and in the nonland grants as well. I think that, as has been indicated, it is very small. It is $16 million right now out of approximately $1.5 billion in agricultural research in total in the country, so it is very small.

Another point that is very important when talking about the competitive grants program and comparing it with the traditional formula funds, is that the biotechnologies that characterize our agriculture today, wheats, corns, animals and so on—and they are biotechnologies—have been largely developed and require long-term commitments of funds. One cannot develop those kinds of technologies in a grants program. It just cannot be done.

If we are going to apply these new techniques of molecular and cellular genetics to the enhancement of agricultural production, I maintain and we maintain it is going to require both an increase, a long-term commitment, in other words, but also the competitive program. There are many advantages to that program but it is infinitesimally small.

Mr. **Gore**. Well, very good. That is great.

One final question, Dr. Brill. We discussed this related issue of the university-corporation relationship in this area. Would you agree that there should be more attention paid by both universities and corporations but particularly universities, to building in safeguards into these new relationships to protect the integrity of the university and the academic environment?

Dr. **Brill**. Yes; I do. I think it is essential that industry does not play a major role, or a given industry playing a major role in governing the way basic research is done. The universities have to worry about that. The Federal Government has to worry about that.

Mr. **Gore**. And should worry about it?

Dr. **Brill**. Should worry about it.

Mr. **Gore**. All right.

Well, you all have gotten us off to a great start. I apologize for keeping you so long. We could ask questions of you all day with the talent on this panel but we have to move on and I would like to again thank you for your contribution here today.

Our second panel is made up of Dr. Nicholas Frey, with Pioneer Hi-Bred International in Johnston, Iowa; Dr. Robert Kaufman, director of agricultural research and cell biology with the Monsanto Corp., accompanied by Dr. John T. Marvel, general manager for agricultural research; and Dr. Sheldon Murphy, a consultant.

Gentlemen, if you would come on up to the witness table, we will get started. Gentlemen, I want to welcome you. As someone who hopes to soon represent a newly configured district that has a large facility owned by Monsanto, I want to give you a special welcome in this election year. [Laughter.]

Mr. GORE. I am told that you have a slide presentation, so why don't we start with you, Dr. John Marvel?

STATEMENT OF DR. JOHN T. MARVEL, GENERAL MANAGER, RESEARCH DIVISION, MONSANTO AGRICULTURAL PRODUCTS CO.

Dr. MARVEL. Thank you, Mr. Chairman. We appreciate the chance to give our thoughts concerning biotechnology to the subcommittee.

We do represent Monsanto Agricultural Products Co., which is an operating company of the Monsanto Co. I think I would like to try to put some perspective, if I can, on the general area, which I do not think will be dissimilar from some of the remarks you have already heard. I think agriculture is quite a complex and broad subject.

Agriculture is a major U.S. industry and we have been blessed with ample, high-quality, arable land which when coupled with our ingenuity, has allowed us to fulfill food needs now and for many years to come. In addition to that, it has of course been a significant economic advantage for the United States, as you have already discussed to some extent.

For example, the 1981 U.S. corn grain crop alone was worth $30 billion and agricultural exports accounted for $44 billion, obviously helping our balance-of-payments situation. The rest of the world, however, is not quite so fortunate. It is estimated or has been estimated that a third of the world's population is living on a marginal diet and some 12 million people starve to death each year.

To make matters worse, United Nations experts expect that the world population will double in the next 35 years. During that time, we must produce as much food as we have already produced since the dawn of recorded history. I think that sets some of the urgency to considering the problem before this committee today.

It will not be a simple task, and it is complicated by the fact that there is very little high-quality agricultural land to bring into production. We have nearly all of the good land that we are going to get, and in our opinion the only way to increase the world food supply is to increase the productivity per unit area of farmland.

Biotechnology offers a number of potential solutions to this problem of food supply but those solutions will not be easy nor immediate. Research in agriculture has been very small compared to that on human health and related areas. A much higher percentage of the money spent on agriculture has been for essential, practical work and basic research has received very limited funding. The result of this spending policy is that there is a woeful lack of knowledge about basic processes in plants, especially biochemistry, physiology, and molecular genetics of plants.

While molecular biologists have succeeded in transforming microbes—introducing and expressing foreign genes—and mammalian cells, plant cells have not yielded to the process yet because we know very little about how genes are organized, controlled, or expressed in plant cells. Unless we are very lucky, much of this knowledge will have to be developed before the technical revolution going on in biology will impact plants and their productivity in a commercial sense.

I would like to emphasize one point on that, that I think that bringing this technology, once you have incorporated it into something in a practical sense to prove that it is valuable to the farmer, is time-consuming and there are some things you cannot do fast in that process.

Mr. GORE. In other words, you are saying the same thing, that plant biotechnology is lagging way behind animal biotechnology.

Dr. MARVEL. Yes, for sure.

Mr. GORE. All right. Go ahead.

Dr. MARVEL. Nonetheless, genetic engineering of plants holds enormous promise for the future. Before discussing this, however, I would like to discuss in general terms some areas that I feel will impact agricultural productivity sooner.

There are a number of factors which will limit crop productivity, including the genetic quality of the crop, the pressure of pests, nutrient ability, and environmental effects. Another aspect of productivity is the regulation of how plants use photosynthesis.

The contributions from biotechnology will probably come first in the area of plant growth regulation. Plants produce grain to ensure species survival but that goal is not necessarily identical to maximum grain yield. Can we regulate the biochemistry of plants to maximize the grain yield of crops? It is not a simple problem, since it integrates research on chemically induced changes in plants, genotypic response to chemical treatment, and environmental effects on chemical-genotypic interactions.

There have been some successes attained in plant growth regulation. There is a commercial product called Pix on cotton, and Polaris on sugarcane, Ethrol on certain other crops. Others should be forthcoming in the major grain crops, soybeans, corn, and wheat, in the next 5 to 10 years.

Chemical solutions to pest pressure problems, including herbicides, insecticides, and fungicides, have significantly increased crop productivity in the period 1940 to 1980. However, these solutions are not perfect and new approaches are needed. Biocontrol, the use of microorganisms to control pests, may provide part of that solution. Microbial pesticides have already been introduced—Dipel, Thuricide, Elcar—and the ongoing revolution in genetic engineering in microbes should lead to improved biocontrol organisms in the future, both in terms of competitiveness and effectiveness.

In addition, antibiotics, which have known applications in agriculture but are too expensive to be widely used at the present time, may be made accessible to farmers by use of recombinant DNA techniques. These areas are being widely studied now by industry and academia and the products should be forthcoming in the next 5 to 10 years.

Improvements in the genetic base of crops, particularly corn, have been dramatic. In fact, the genetic improvements in corn in conjunction with the use of chemical fertilizers have afforded yield increases of greater than 300 percent since 1940. Nevertheless, due to flower morphology many crops, including soybeans, wheat, rice, and cotton, remain difficult to improve and novel approaches are needed.

Plants have a remarkable property in that their cells can be cultured, which you heard from Dr. Mott earlier this morning—grown on a synthetic diet and subsequently regenerated into whole plants. The potential of this technology is that plant cells may be selected in culture for new traits, such as herbicide resistance, disease resistance, lysine production, et cetera, and more rapidly at the cellular level than in the whole plant. Hence, tissue culture technology may help introduce new plant types more rapidly.

I also should add that if this technology is not in place it will be very difficult to utilize recombinant DNA technology, for if you cannot develop protoplasts into whole plants in commercially useful crops, it has a severe limitation on what can be done with recombinant DNA.

Finally, when scientists learn how to effectively transform plants, tissue culture will be used to install new traints into the plants directly to facilitate genetic improvements in crops even more rapidly. Tissue culture and recombinant DNA will probably not impact crop improvement for 10 to 15 years, barring unforeseen breakthroughs.

While we are very enthusiastic about the potential of biotechnology, let me summarize some of the important reasons for the time delay: The lack of fundamental biochemical knowledge in plant sciences' plant transformation is not yet feasible; the lack of evidence that tissue culture can produce competitive commercial plants; the lack of evidence that new traits can be incorporated successfully into new plants and their seed; and the new variety introduction now is an 8- to 12-year process and it probably will not change significantly, if at all, for 5 to 10 years.

The final word is research which is needed in plants. While the United States is a leader in basic and applied plant research, this area has been given too little emphasis in the past, undoubtedly due to our fortunate climatological and geographic circumstances. I would like to add one comment to that, though, in reference to the article you mentioned. The USDA and the Department of Agriculture, I do not think should be singled out, as it has produced the best agricultural system in the world to date.

However, food or the lack of it will surely become one of the major destabilizing issues in the next 30 years in the world. Further, the present economic plight of the farmer emphasizes the need to make him cost efficient and resource efficient, consistent with better national policies on water utilization and land utilization. The United States must meet this challenge of feeding the world in the future.

[The prepared statement of Dr. Marvel follows:]

STATEMENT OF DR. JOHN T. MARVEL, PH.D., GENERAL MANAGER, RESEARCH DIVISION, MONSANTO AGRICULTURAL PRODUCTS COMPANY, BEFORE THE SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHTS OF THE HOUSE COMMITTEE ON SCIENCE AND TECHNOLOGY, JUNE 9, 1982

Mr. Chairman: It is an honor today to present our thoughts concerning biotechnology to the Subcommittee. We represent Monsanto Agricultural Products Company, an operating company of Monsanto Company with $6,947,000,000 sales in 1981, $1,216,000,000 of which was Agricultural Products sales.

Agriculture is a major United States industry. We have been blessed with ample high quality arable land which, when coupled with American ingenuity, has allowed us to fulfill United States food needs now and for many years to come. In addition to fulfilling our food needs, agriculture provides a significant economic advantage for the U. S. The 1981 U. S. corn grain crop alone was worth $30 billion dollars, and agricultural exports accounted for $44 billion dollars, obviously impacting our balance of payment situation quite favorably.

The rest of the world has not been so fortunate. In fact, it is estimated that one-third of the world population is living on a marginal diet and that some 12 million people starve to death each year. To make matters worse, United Nations experts expect that the world population will double in the next 35 years. During that time we must produce as much food as we have already produced since the dawn of recorded history!

This will be no simple task and it is complicated by the fact that there is very little high quality agricultural land to bring into production. We have all the good land that we are going to get and, in our opinion, the only way to increase the world food supply is to increase the productivity per unit area of farm land.

Biotechnology offers a number of potential solutions to this problem of food supply, but these solutions will not be easy nor immediate. Research in agriculture has been a meager $1,540,000,000 compared to $3,836,000,000 on human health and related areas. A much higher percentage of the monies spent on agriculture have been for essential, practical work, and basic research has received very limited funding. The result of this spending policy is that there is a woeful lack of knowledge about basic processes in plants, especially the biochemistry, physiology and molecular genetics of plants.

While molecular biologists have succeeded in transforming microbes (introducing and expressing foreign genes) and mammalian cells, plant cells, have not yet yielded to the process because we know very little about how genes are organized, controlled or expressed in plant cells. Unless we are very lucky, much of this knowledge will have to be developed before the technical revolution going on in biology will impact plants and their productivity in a commercial sense.

Nonetheless, the genetic enginering of plants holds enormous promise for the future. Before discussing this, however, I would like to discuss in general terms some areas that I feel will impact agricultural productivity much sooner.

There are a number of factors which limit crop productivity including the genetic quality of the crop, the pressure of pests, nutrient ability and environmental effects. Another aspect of productivity is the regulation of how plants utilize photosynthesis.

The contributions from biotechnology will probably come first in the area of plant growth regulation. Plants produce grain to insure species survival, but that goal is not necessarily identical to maximum grain yield. Can we regulate the biochemistry of plants to maximize the grain yield of crops? This is not a simple problem since it integrates research on chemically induced changes in plants, genotypic response to chemical treatment and environmental effects on chemical-genotypic interactions.

There have been some successes already attained in plant growth regulation (e.g., Pix in cotton and Polaris in sugarcane). Others should be forthcoming in the major grain crops, soybeans, corn and wheat, in the next 5-10 years.

Chemical solutions to pest pressure problems, including herbicides, insecticides and fungicides, have significantly increased crop productivity in the period 1940-1980. However, these solutions are not perfect and new approaches are needed. Biocontrol, the use of microorganisms to control pests, may provide part of that solution. Microbial pesticides have already been introduced (e.g., Dipel, Thuricide, Elcar), and the ongoing revolution in genetic engineering of microbes should lead to improved biocontrol organisms in the future, both in terms of competitiveness and effectiveness. In addition, antibiotics, which have known applications in agriculture but are too expensive to be widely used, may be made accessible to farmers by use of recombinant DNA techniques. These areas are being widely studied now by industry and academia, and products could be forthcoming in 5-10 years.

Improvements in the genetic base of crops, particularly corn, have been dramatic. In fact, the genetic improvement in corn, in conjunction with the use of chemical fertilizers, have afforded yield increases greater than 300% since 1940. Nevertheless, due to flower morphology, many crops, including soybeans, wheat, rice and cotton, remain difficult to improve. Novel approaches are needed.

Plants have a remarkable property in that their cells can be "cultured", i.e., grown on a synthetic diet, and subsequently regenerated into whole plants. The potential of this technology is that plant cells may be selected in culture for new traits, e.g., herbicide resistance, disease resistance, lysine production, etc., and more rapidly at the cellular level than at the whole plant level. Hence, tissue culture technology may help introduce new plant types more rapidly.

Finally, when scientists learn how to effectively transform plant cells, tissue culture will be used to install new traits into plants directly to facilitate genetic improvement in crops even more rapidly.

Tissue culture and recombinant DNA will probably not impact crop improvement for 10-15 years, barring unforeseen breakthroughs.

While we are very enthusiastic about the potential of biotechnology, let me summarize some of the important reasons for the time delay:

- the lack of fundamental biochemical knowledge in plant sciences;
- plant transformation is not yet feasible;
- the lack of evidence that tissue culture can produce competitive commercial plants;
- the lack of evidence that new traits can be incorporated successfully into plants;
- new variety introduction is an 8-12 year process now and probably will not change significantly, if at all, for 5-10 years.

The final word is research. Much is needed in plants. While the United States is a leader in basic and applied plant research, this area has been given too little emphasis in the past, undoubtedly due to our fortunate climatological and geographic circumstances. However, food or the lack of it will surely become one of the major de-stabilizing issues in the next 30 years. Furthermore, the present economic plight of the farmer emphasizes the need to make him cost efficient and resource efficient consistent with better national policies on water utilization and land utilization. The United States must meet this challenge of feeding the world in the future. Thank you.

Mr. GORE. Thank you very much.

To introduce our next witness, Dr. Kaufman, who is also with Monsanto, I would like to defer to my colleague.

Mr. VOLKMER. Thank you very much, Mr. Chairman.

I wish to welcome both Dr. Kaufman and Dr. Marvel here today. We in Missouri are very proud of the work that Monsanto has been doing on plant biotechnology. Perhaps I could mention the fact that a little over a year ago I and some other people visited the research plant there in St. Louis, Monsanto, and were quite impressed with the work that is being done. I am sure that if the chairman would wish, the opportunity may be available for him and some of the staff to go there and see the actual work that is being done. It is quite impressive.

Mr. GORE. Well, we may do that.

Thank you.

Dr. Kaufman.

STATEMENT OF DR. ROBERT J. KAUFMAN, RESEARCH DIRECTOR, PLANT SCIENCES, MONSANTO AGRICULTURAL PRODUCTS CO.

Dr. KAUFMAN. Thank you, Mr. Chairman. It is an honor and a pleasure for me to discuss with you today some of the areas where I think biotechnology will have a significant impact on agriculture.

Jack Marvel has talked about several areas, improved microbiologicals, plant growth regulation, and tissue culture and recombinant DNA for its potential in increasing crop yields. If I may, I think it might be useful to expand on the tissue culture and genetic engineering areas, especially using some slides to graphically illustrate the kind of things we are talking about. May I proceed up to the projector?

Mr. GORE. Fine.

Dr. KAUFMAN. Plant breeders over the last 40 or 50 years have made outstanding gains in the crop yields in the United States, particularly in corn. The reason for these gains, and particularly in corn, has to do with the flower morphology.

If you look at the corn plant in this picture, the male reproductive organ is the tassle and the silks down on the ear are the female part, and you can see the parts of the flowers are widely separated. This allows the breeder to make a specific cross and it also lends itself to hybrid seed production. Now corn amongst plants is unique in this regard, and this unique flower morphology has allowed for about a 300-percent increase in grain yield over the past 40 years, in conjunction with the use of high nitrogen fertilizers. You can see inbred lines, which are the ears on the right and the left, compared to the ear that one gets from the hybrid corn plant.

If you look at a flower of other plants, this is a soybean flower. The yellow is the male part and the white is the female part. You can see they are very close together. It is very hard to separate them and crosses and hybrid seed production are very, very difficult. Therefore, what we would like to do with tissue culture is to obviate the use of the sexual process or at least relegate it to a secondary role.

Therefore, in this regard I am going to be reproducing some of Dr. Mott's remarks earlier on the potential benefits of tissue culture. Dr. Mott mentioned the totopotency of cells, that is, that any given plant cell has ability to not only make the whole plant back but any plant part. Therefore, if you had a general plant cell, it contains the genes for making roots, it contains the genes for making leaves, and in the case of a cotton plant illustrated here, it contains the genes for making the cotton boll.

This gives us an amazing potential but even more important, plants can be regenerated from these cells, and this is something that is unique in biology. I have put some slides here to illustrate the process. If you take a cutting from a plant such as alfalfa, you can put that, those plant parts onto a synthetic media and this synthetic media provides the nutrients, the carbohydrates, the fertilizer, and the minerals for these materials to grow.

As they grow they begin to lose the characteristics of the tissue from which they were derived and they begin to resemble dedifferentiated tissue, which is called callus. Callus, in a closeup, looks like that. You can see it does not look much like leaves at all.

You begin to juggle the hormonal composition of that callus and you can begin to get plants to grow back from that callus. Here is an example of an alfalfa plant which has now been regenerated from a piece of callused tissue. You can take this and plant it out in the greenhouse and ultimately out in the field and you have back the alfalfa plant that you started out with.

The advantages, though, of being able to go through tissue culture are, as Dr. Mott alluded to earlier, you can put thousands and thousands of cells into a very small area and you can begin to select for different types of cell variants, either through selection processes where you try to poison the cells or just simply through the variation that is available from going through tissue culture itself. I will show you now an example of a system that we have done at Monsanto.

We started out by putting some of these callus cells or clumps into a liquid culture which breaks them up into smaller clumps, almost down to single cells. Then we can expose them to a herbicide at a level that would normally be toxic to the plant itself and to these cells in particular, and then you can plate these cells out onto a solid media and ask the question, "Who amongst the cells that I put into this liquid system have survived?"

As you can see, most of the cells do not grow. They have been killed by the herbicide but one of the clumps of cells has regrown into a callus. You can juggle the hormones again and cause this to bud and start to form shoots and ultimately you can get plants back out in the field, and you can spray herbicides on them just like a farmer would to see if these things have worked and if you have really been successful.

In fact, we have done this with alfalfa and we put the plants into culture, tested them against the herbicide Round-Up, regenerated the survivors, took them out to the field, sprayed them with Round-Up, and found that some of them survived so we were successful in getting some variants.

As a consequence, we also evaluated what was the effect of putting these plants into culture on their agronomic characters: Do they stand up in the field like they should? Do they yield like they should? Do they have the same disease resistance characters that they had when we put them into culture? We found that in many cases these things had remained satisfactory as well but with alfalfa, this is not a short-term project. You cannot do it one summer. You have to do it 3, 4, or 5 years to make sure that the quality of the plant has been maintained.

Now there are a lot of disadvantages to tissue culture, one of which is, things go into culture and come out different than they started out. There are genetic changes going through culture, and sometimes the changes you get are not changes you want. You get things in addition to the changes that you asked for.

Therefore, this brings us to the potential utility of recombinant DNA. Genetic engineering allows you in principle to put a single trait into a plant, and to do so, in theory, clearly. This brings us to the central dogma of molecular biology which is, you have DNA which is the genes. DNA codes for RNA which in turn codes for proteins. Proteins are the workhorses of the cell. For instance, there are proteins involved in taking that energy from the Sun and fixing CO_2 and turning it into sugars, which in turn fuel the whole plant's biochemical system.

Well, there are thousands of proteins in a plant cell, all of which have different characters. Some are for disease resistence, some are for herbicide resistance, some are for stress tolerance, some are structural; and on and on and on. If one can identify the gene that is of interest, then you can presumably move that around in plant cells. The idea of transformation is demonstrated here in microbes.

You can get a gene, for instance, in some microorganisms that codes for antibiotic resistance. You can isolate that gene from the organism and then you can either synthesize the gene or you can clone it up and get many, many copies of it. Then you can put that gene back into a microbe, which is the one depicted on the right, that lacks resistance to the antibiotic, and that effectively will transform a susceptible bacteria into an antibiotic resistant bacteria.

This is the same kind of thing people would like to do with plants. I am going to skip over this stuff because I do not think it is that relevant but if you had a gene on a plasmid that could code for herbicide resistance, you could put this gene into a plant cell and get it integrated and expressed. Then the plant cell that contains that gene would be able to be resistent to herbicides, whereas the one that did not have that gene would not. Then the consequence would be, when you treated it with a herbicide, the one that has the gene lives, the one that does not have it dies.

An additional advantage of this type of technology is, if you have a gene that is not a life or death matter from the plant, that merely improves the quality, you could put that gene in tandem with the herbicide-resistant gene and that allows you to select so that you can get both genes expressed into the plant at the same time.

We have been talking about cloning all day. I just wanted you to know that there was a lamppost at Monsanto that cloned itself in about 1970, along before all this publicity started. [Laughter.]

Anyway, that is all the slides I have to show. Of course, as everyone has told you today, it is still impossible to apply genetic engineering to plants because no one has successfully transformed a plant cell.

Even worse than that, we all agree I think that someone will in fact transform a plant and they will get a foreign gene expressed in there but then what are they going to do with it? Expression of genes, regulation of genes, things of this nature are just not understood in plant cells at all.

A good example of how this has gone awry is, suppose you had the genes that could make high lysine and you could put that into a corn plant. If you put that into a single plant cell and you regenerate that into a whole corn plant, you would have lysine made in the roots, the stem, the leaves, and the grain, and the only place you would really want it is in the grain.

Therefore, somehow we have to learn to put genes in and get them turned on, at the time we want them turned on, or we are going to waste a lot of time and money doing all this stuff. This gets back to what you have been hearing all day—and I am sorry to sound like a stuck record—but we really need more basic research in plant biochemistry, plant physiology, and plant molecular genetics.

Thank you.

[The prepared statement of Dr. Kaufman follows:]

STATEMENT OF DR. ROBERT J. KAUFMAN, PH.D., RESEARCH DIRECTOR, PLANT SCIENCES, MONSANTO AGRICULTURAL PRODUCTS COMPANY, BEFORE THE SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHTS OF THE HOUSE COMMITTEE ON SCIENCE AND TECHNOLOGY, JUNE 9, 1982

Mr. Chairman: It is an honor for me to discuss with you today the potential impact of biotechnology on agriculture. There are several areas of biotechnology which may impact agricultural productivity in the next 5-25 years, including plant growth regulation, microorganisms for biocontrol of pests, and crop improvement through tissue culture and genetic engineering of plants. Tissue culture and genetic engineering, while furthest away from implementation, hold the greatest promise as breeding tools to improve crops and productivity. It may well be twenty-five years before this technology fully impacts the American farmer.

Plant breeding, particularly in corn, has greatly improved the quantity and quality of crops in the U. S. and the world. The reason that corn has been particularly impacted is that the "flower" of the corn plant readily lends itself to breeding and hybrid production. The male part of the corn flower (tassel) is far removed from the female part (silks), allowing specific crosses to be made without self-fertilization. Corn is also readily emasculated, which makes corn conveniently adapted to hybrid seed production. Hybrid vigor in corn in conjunction with nitrogen fertilizer use had led to a 300% increase in corn yield since the 1940's. In contrast, the flowers of soybeans, rice, wheat and cotton have the male and female parts almost in contact, making crosses tedious and difficult and virtually excludes hybrid seed production. Plant tissue culture and genetic engineering offer the potential to circumvent the natural limitations of flower morphology in crops such as soybeans, rice, wheat and cotton.

Plant tissue culture is the growth of plant parts such as leaves, stems, roots, embryos, etc. on synthetic media. Under these conditions a plant obtains nutrients, minerals and sugars from the media instead of from the soil and photosynthesis. Under culture conditions, plant tissue generally becomes disorganized into callus. Callus may be cultured as is or broken into clumps of cells. In the presence of appropriate plant hormones, callus from some plants can be induced to undergo morphogenesis or regeneration into whole plants. It is this remarkable property which makes plant tissue culture a potential tool for crop improvement. Literally millions of plant cells can be grown in culture in a small room. These cells can be selected for new properties such as protein quality, herbicide resistance and disease resistance. Variants may be selected, regenerated and evaluated at the whole plant level in the greenhouse or field. Hence, selections for new plant types which would require large areas of land and many years might be accomplished more rapidly in tissue culture.

An example of this type of technology which has recently been completed at Monsanto is the development of Roundup® resistant alfalfa plants. Alfalfa tissue was placed into culture first on solid media and later into liquid culture. In liquid culture, the cells were exposed to a lethal dose of the herbicide Roundup® and the survivors were plated out for regeneration into whole plants. These new plants (or variants) were transplanted into the field and treated with Roundup® the way a farmer would use the herbicide. Several variants were found to have field resistance to the herbicide. In addition, the other agronomic characters of the plant - standability, morphology, and disease resistance - appeared to be unchanged by the selection and regeneration process. The evaluation process is by no means complete and several more years of field work will be necessary before this experiment can be declared a complete success. Nonetheless, this illustrates the potential utility of this technology for crop improvement.

There still remain a number of problems with the use of tissue culture. First and foremost, not all crops can be regenerated from tissue culture. Most notable amongst those which cannot be regenerated are soybeans and cotton while wheat is regenerable only under special circumstances. Certain crops which are regenerable undergo dramatic changes during culture and may lose many desirable agronomic traits during the process. Potatoes are such a crop. Most crops cannot be regenerated from single cells and selections from clumps of cells may produce highly heterogeneous regenerates which will be difficult to use in a breeding program. Furthermore, selection in culture for e.g. herbicide resistance may result in variants with many changes besides the ones sought.

Finally, genetic transmission of tissue culture induced traits through the seed does not always occur. Considerable applied and basic research will be required to fully exploit the potential of tissue culture for crop improvement.

Genetic engineering offers an extremely selective method for modifying plants in culture. Instead of selecting for desirable traits by screening sheer numbers of cells for mutants (or variants), the gene coding for the desired trait is placed into the cell. The resulting transformed cell now codes directly for the desired trait. For example, genes coding for antibiotic resistance have been inserted into antibiotic susceptible bacteria, transforming these bacteria to antibiotic resistance organisms. Similarly, if a gene coding for herbicide resistance were available, it could theoretically be inserted into a plant cell. This transformed plant cell could be regenerated into a whole plant which would putatively be resistant to herbicides. The potential advantage of genetic engineering is that a single gene change can be made in a plant cell as opposed to the unpredictable number of changes which may occur while selecting variants in culture. Unfortunately, plant cells have yet to be transformed and until this breakthrough occurs, genetic engineering of plants cannot be accomplished. While simple transformation of plant cells will probably occur in the next one to three years, useful transformation will require considerable research into gene organization, regulation and expression in plant cells.

Until this research has been completed, it will be very difficult to place a gene into a plant cell and obtain meaningful expression at the right time and place. For example, if the gene coding for lysine were simply inserted into a corn cell and this cell regenerated into a whole plant, this plant might produce high quantities of lysine in all tissues (e.g. leaves, roots, stem). Since high lysine is only desirable in the grain, it is obvious that considerable energy would be wasted by the plant in producing lysine in all tissues.

Despite all the technical problems of tissue culture and genetic engineering, these technologies hold the key to revolutionizing plant breeding and crop productivity.

More research on plant biochemistry, physiology and molecular genetics needs to be undertaken in University, Industry and Government labs to speed up the realization of this potential for improved crop productivity. Thank you.

Mr. GORE. Before we can predict the time of these big impacts?

Mr. KAUFMAN. Exactly. Now you cannot rule out that someone will have a major breakthrough quite by accident.

Mr. GORE. Yes.

Mr. KAUFMAN. I mean, it has happened time and time again. People did not understand physics but they got planes off the ground. Therefore, it may happen but it probably is not going to.

Mr. GORE. OK.

Dr. Nicholas Frey with the Pioneer Hi-Bred from Johnston, Iowa, welcome. We will be delighted to hear from you. Please proceed.

STATEMENT OF DR. NICHOLAS M. FREY, COORDINATOR OF JOHNSTON RESEARCH, PIONEER HI-BRED INTERNATIONAL, INC.

Dr. FREY. Thank you. It is a pleasure to be here.

Pioneer Hi-Bred International is a seed company that develops proprietary seeds through conventional plant breeding, conventional genetic applications. These seed stocks are produced and then sold directly to farmers. I will confine my comments in general to plants, the seed stocks that our company would be involved with.

Biotechnology will likely impact our business at the product development or plant-breeding level. The questions then become how and when biotechnology will affect crop productivity and plant breeding.

Plant breeding is a primitive form of genetic engineering that exploits favorable genetic recombination to improve crop productivity. The breeder has to rely on the science of statistics—that is, the laws of probability—to identify genetically superior varieties and hybrids. This approach has resulted in continuous improvements in the yield of our major crops over the past several decades. Genetic improvements in corn yields over the past 50 years have averaged approximately 1.5 bushels per acre per year, merely due to genetics, based upon research by Dr. Donald Duvick of Pioneer. This separates out the technology advances such as nitrogen fertilization.

I might state at this point as well that in our data on corn, and if you plot national corn yield averages, we do not see a leveling off in productivity. Yields are continuing to increase. We are investing more and more money to try to maintain that same rate, however. More effort is going into achieving those gains.

Mr. GORE. Have the percentage productivity gains been smaller in recent years?

Dr. FREY. Well, when you have a constant 1.5 bushels per acre per year increases, I guess you would say that the percentage gain is declining slightly due to the higher mean yields. You cannot predict into the future on regression statistics, but at least the past still indicates a linear increase in yield.

Plant breeding has been defined as directing the accelerated evolution of crop plants. Evolution implies a continuous change over time. Plant-breeding improvements have been incremental. There have been no large, single generation increases in productivity of 50 or 100 percent.

A plant is a complex organism, sufficiently complex that major shifts in productivity have not occurred. Yields are not often limited by a single trait but a series of traits, dozens of traits. As I make one trait nonlimiting, I identify another limitation. Genetic improvements by plant breeding are slow but again, they have been continuous.

How does this relate to biotechnology and its potentials for crop improvement? I see biotechnology as a means to better direct the accelerated evolution of crop plants, to do a better job of breeding. I still envision continuous increments in crop improvement, not great leaps in performance of 50 or 100 percent. Furthermore, improvements in plant performance through genetic engineering will not come as easily or quickly as they will in microbial performance or in human health because the knowledge base of plant function and genetics lags behind that of mammilian and microbial systems.

Funding for basic plant research has lagged those other areas. A lack of a firm foundation of basic knowledge in plant function will make biotechnology gains more difficult. Biotechnology accentuates the need for greater investments in research from the whole plant level all the way down to the single cell level and finally to the molecular level. We cannot likely translate subcellular changes the molecular geneticist makes into yield improvements on 14 million acres of corn in Iowa if we have large gaps in our knowledge of how a corn plant develops its yield. Plant research needs to remain in balance to achieve maximum productivity, just like a plant's physiology must remain in balance if it is to achieve maximum productivity.

Genetic engineering of plants may be more difficult than engineering insulin production but that does not mean progress will be slow. The tremendous investment in plant molecular genetics will result in significant advances. First, knowledge of gene function and regulation will help the breeder improve his techniques and effectiveness. Second, simply inherited traits will be more quickly incorporated into crop plants to meet specific needs.

Resistance or tolerance to specific diseases can often be achieved by inserting a single gene. Plant breeders take 5 or more years to achieve this through the breeding technique called backcrossing. If a molecular geneticist and tissue culture specialist could achieve this in the laboratory in a few months, the breeder could avoid backcrossing. I personally feel such complementation between the molecular geneticist and the plant breeders must occur if the molecular techniques are to be translated into improved crop productivity.

I also feel that genetic engineering may allow us to broaden the range of superior genotypes by correcting those small flaws that limit yield in a given area. For example, today Pioneer cannot afford to develop a corn breeding program to improve the salt tolerance of corn in irrigated regions where salinity is becoming a problem. As soon as we restrict a breeding program by applying maximum selection pressure for salt tolerance—or it might be white endosperm or virus tolerance—we restrict the genetic base with which to work. As a result, yield gains are slowed. Breeders working without that restriction, whatever it may be, make more rapid gains in performance. Soon this specialty breeding program lags hopelessly behind the yield performance even if the salt problem is overcome. The products of that specialized breeding program only work on a limited acreage—in other words, only where you have salt problems. Products from our other breeding programs have potential of working anywhere that corn is grown—they usually do not but they have that potential.

Perhaps genetic engineering can take care of those special restrictions in productivity, thus allowing farmers to grow the most advanced genetic materials even if they have a limiting factor such as salt or virus to contend with. This hope for genetic engineering could have significant effects on national average yields by trimming off the lower end of the yield spectrum and maybe having no effect on the upper end of that spectrum. Profits from such improvements will not likely support current private biotechnology investments, however.

What will support the biotechnology investment? Today I do not know. A Batelle report for the EPA listed 12 areas where agriculture would likely benefit. Their key point in my opinion was their 12th that stated biotechnology would expand the basic knowledge of gene structure, function, and regulation including molecular mechanisms that control productivity. All of our gains in agriculture today have been achieved without that understanding.

For Pioneer, knowledge of gene structure, function, and regulation will surely help our breeders be more effective. If our breeders are effective, our product line will remain competitive and our company will remain successful, but our accountants will have a difficult time calculating the return on investment of biotechnology research.

I am sure new products and perhaps new crops will emerge as a result of biotechnology advance. When those changes will occur is difficult to predict. Plant tissue culture will make and is making the first contributions to improve crop productivity. Today tissue culture specialists are utilizing the same principles of probability that plant breeders use in selecting superior genotypes. In the future they will team with the molecular geneticist to engineer specific changes.

How significant genetically engineered agricultural advances become will depend in part upon how much resistance is encountered in the marketplace. For example, if I genetically engineer a high-yielding wheat—let's say it is a 100- percent yield gain—but the millers cannot use that in flour, how big of an impact will that have in the marketplace? The market chain may temper the successful commercial development of new genetically engineered crops or crop products.

Biotechnology will not soon force radical shifts in our cropping systems. Much research will be needed and that will take time and money. The potential benefits of that research far outweigh any risks that I envision. The goal of this research is to benefit crop production. Contributions by biotechnology will accrue most rapidly if we focus on the real potentials of the technology rather than forcing our creative thoughts through an unreasonably restrictive atmosphere to control potential hazards.

In summary, I look forward to the revolution genetic engineering will bring. I feel the greatest revolution will be in the basic knowledge of gene structure, function, and regulation, including the molecular mechanisms that control plant productivity. That revolution will be needed if we are to maintain or increase our current rates of gain in productivity.

Furthermore, we must keep our research in balance. Biotechnology does not replace any facet of research and certainly does not replace plant breeding. Instead, biotechnology augments the entire research effort needed to improve agricultural productivity. Biology as well as research is a complex system. There are few shortcuts to success or exponential leaps forward. Instead, a continual evolution is more likely required, and our greatest opportunity to successfully enhance the system is to enhance the rates of those evolutionary processes.

Thank you.

[The prepared statement of Dr. Frey follows:]

Genetic Engineering of Crop Plants

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Plant Breeding has been defined as directing the accelerated evolution of crop plants. Evolution implies a continuous change over time. Plant breeding improvements have been incremental. There have been no large, single generation increases in productivity of 50 or 100 percent. A plant is a complex organism, sufficiently complex that major shifts in productivity have not occurred. Yields are not often limited by a single trait, but by a series of traits - dozens of traits. As I make one trait nonlimiting, I identify another limitation. Genetic improvements by plant breeding are slow, but they have been continuous.

How does this relate to biotechnology and its potentials for crop improvement? I see biotechnology as a means to better direct the accelerated evolution of crop plants. I still envision continuous increments in crop improvement, not great leaps in performance of 50 or 100 percent. Furthermore, improvements in plant performance through genetic engineering will not come as easily or quickly as they will in microbial performance or in human health because the knowledge base of plant function and genetics lags behind that of mammalian and microbial systems. Funding for basic plant research has lagged greatly relative to those areas. Lack of a firm foundation of basic knowledge in plant function will make biotechnology gains more difficult. Biotechnology accentuates the need for greater investments in research from the whole plant level all the way down to the single cell level, and finally to the molecular level. We cannot likely translate subcellular changes the molecular geneticist makes into yield improvements on 14 million acres of corn in Iowa if we have large gaps in our knowledge of how a corn plant develops its yield. Plant research needs to remain in balance to achieve maximum productivity just like a plant's physiology must remain in balance if it is to achieve maximum productivity.

Genetic engineering of plants may be more difficult than engineering insulin production, but that does not mean progress will be slow. The tremendous investment in plant molecular genetics will result in significant advances. Firstly, knowledge of gene function and regulation will help the breeder improve his techniques and effectiveness. Secondly, simply inherited traits will be more quickly incorporated into crop plants to meet specific needs. Resistance or tolerance to specific diseases can often be achieved by inserting a single gene. Plant breeders take 5 or more years to achieve this through the breeding technique called backcrossing. If a molecular geneticist and tissue culture specialist could achieve this in the laboratory in a few months, the breeder could avoid backcrossing. I personally feel such complementation between the molecular geneticists and the plant breeders must occur if the molecular techniques are to be translated into improved crop productivity.

I also feel that genetic engineering may allow us to broaden the range of superior genotypes by correcting those small flaws that limit yields in a given area. For example, today Pioneer cannot afford to develop a breeding program to improve the salt tolerance of corn in irrigated regions where salinity is becoming a problem. As soon as we restrict a breeding program by applying maximum selection pressure for salt tolerance, or white endosperm, or virus tolerance, we restrict the genetic base with which to work. As a result, yield gains are slowed. Breeders working without that restriction make more rapid gains in performance. Soon this specialty breeding program lags hopelessly behind in yield performance even if the salt problem is overcome. The products of that specialized breeding program only work on a limited acreage. Products from our other programs have potential of working anywhere corn is grown. Perhaps genetic engineering can take care of those special restrictions in productivity, thus allowing farmers to grow the most advanced genetic materials even if they have a limiting factor such as salt or virus to contend with. This hope for genetic engineering could have significant effects on national average yields by trimming off the lower end of the yield spectrum. Profits from such improvements will not likely support current private biotechnology investments, however.

What will support the biotechnology investment? Today, I do not know. A Batelle report for the EPA listed twelve areas where agriculture would likely benefit. Their key point in my opinion was their twelfth that stated biotechnology would expand the basic knowledge of gene structure, function, and regulation, including molecular mechanisms that control productivity. For Pioneer, knowledge of gene structure, function, and regulation will surely help our breeders be more effective. If our breeders are effective, our product line will remain competitive and our company will remain succesful. But our accountants will have a difficult time calculating return on investment in biotechnology research.

I am sure new products and perhaps new crops will emerge as a result of biotechnology advance. When those changes will occur is difficult to predict. Plant tissue culture will make (is making) the first contributions to improved crop productivity. Today tissue culture specialists are utilizing the same principles of probability that plant breeders use in selecting superior genotypes. In the future they will team with the molecular geneticist to engineer a specific change. How significant genetically engineered agricultural advances become will depend in part upon how much resistance is encountered in the market system. For example, if I genetically engineer a high yielding wheat (100 percent yield increase), but the millers will not accept it for flour, how big will the impact be? The market chain may temper the successful commercial development of new genetically engineered crops or crop products.

Biotechnology will not soon force radical shifts in our cropping systems. Much research will be needed and that will take time and money. The potential benefits of that research far outweigh any risks that I envision. The goal of this research is to benefit crop production. Contributions by biotechnology will accrue most rapidly if we focus on the real potentials of the technology rather than forcing our creative thoughts through an unreasonably restrictive atmosphere to control potential hazards.

In summary, I look forward to the revolution genetic engineering will bring. I feel the greatest revolution will be in the basic knowledge of gene structure, function, and regulation including the molecular mechanisms that control plant productivity. That revolution will be needed if we are to maintain or increase our current rates of gain in productivity. Furthermore, we must keep our research in balance. Biotechnology does not replace any facet of research and certainly does not replace plant breeding. Instead biotechnology augments the entire reserach effort needed to improve agricultural productivity. Biology as well as research is a complex system. There are few shortcuts to success or exponential leaps forward. Instead a continual evolution is more likely required, and our greatest opportunity to successfully enhance the system is to enhance the rates of those evolutionary processes.

Nicholas M. Frey

Presented to the Subcommittee for Investigation and Oversight of the House Committee for Science and Technology, June 9, 1982.

Specific Considerations For The Application Of Biotechnology to Crop Improvement

I. Factors of primary concern to plant breeders are controlled by several (many) genes, e.g., yield, standability, stability of performance under stress, grain drydown in the field.

Genetic engineering will be useful on single gene traits or traits controlled by only a few genes.

II. Products developed in the laboratory through tissue culture selection or through gene insertion will all have to be evaluated for performance in the field.

Field evaluation will determine if the desired trait(s) is expressed, if undesired changes occurred, and what the environmental range of adaptation is.

If undesired changes occur, the materials will have to be reselected by plant breeders using conventional techniques.

III. For biotechnology to gain widespread usage, techniques must work on a large number of genotypes within a given crop species, especially modern, high yielding genotypes.

IV. Potential biotechnology contributions should be assessed based upon the laws of nature, i.e. biotechnology cannot create energy. Greater output from the agricultural system will require greater inputs or increased efficiency in partitioning resources to harvestable yield. For example, nitrogen fixation by corn would be an energy requiring process. Energy used to fix nitrogen is not available for harvestable yield. Economics will determine if genetically engineered nitrogen fixation by grasses would be desirable compared to commercial nitrogen application.

Nicholas M. Frey
Pioneer Hi-Bred International, Inc.

DR. NICHOLAS M. FREY
Coordinator of Johnston Research
Pioneer Hi-Bred International, Inc.

Dr. Nick Frey joined the Plant Breeding Division in 1974 and served as research plant physiologist for the division for six years. In August, 1980 he was named Johnston Research Coordinator.

Frey, who was raised in Iowa, attended Iowa State University, where he received his B.S. degree in agronomy in 1970. He then attended the University of Minnesota, earning his Ph.D. degree in plant physiology in 1974. He was recipient of the Goke Estate Scholarship in 1967 and Caleb Dorr Fellowship in 1973.

Frey is a member of the American Society of Agronomy, the Crop Science Society of America, the American Association for the Advancement of Science and the American Society of Plant Physiologists. He served as advisory editor of the latter organization's journal for two years and is on its Review Board.
(Birth date: April 14, 1948)

Mr. GORE. Thank you for an excellent statement.

Our final witness on this panel is Dr. Sheldon Murphy, the leading consultant in this area. Dr. Murphy, we are privileged to have you here. Please proceed.

STATEMENT OF DR. SHELDON R. MURPHY, CONSULTANT, THE PERFORMANCE MANAGEMENT GROUP, NEW CANAAN, CONN.

Dr. MURPHY. I am not sure I am the leading consultant in this area but I am pleased to be here. I will address my comments to the area of agricultural pesticides, specifically insecticides, with regard to the potential for biotechnology to influence these.

In North America there are about 700 species of insects and other arthropods that are considered pests and have a substantial impact on the productivity and the yield of crops grown in the field. Dr. Marvel mentioned the magnitude of U.S. crop values in the United States. These are multiplied, of course, when one considers the crop values worldwide, yet it has been estimated that between a fourth and a third of this crop potential is lost in the field every year due to pest damage, insect damage.

Modern technologies rely heavily on the use of chemicals to control these pests. This practice of near sole reliance on chemical insecticides for insect and mite control has been in a sense unsuccessful due to the attendant problems of resistance of target pests, increasing need for more pesticides, mounting costs for pest control, the reduction of natural enemies, and the impact of chemical pesticides on the environment. There is also ample evidence that broad spectrum—that is, insecticides that kill a large range of insects—have results in explosions of secondary pests which are not well controlled by that chemical, requiring additional chemical treatment. About 200 incidents of resistance have now been known and some pests are literally impossible to control any longer with chemical insecticides. Furthermore, the influence of these compounds on the environment is of great concern to the American public.

The effects of pesticides on the environment and on human health are difficult to assess quantitatively but certainly certain chemicals such as DDT and Kepone and Leptophos are unacceptable, as evidenced by recent rulings that have removed them from use.

The losses attributed to insects, as well as the increased cost of control techniques, have caused concern for the future. Dr. Marvel mentioned the increasing requirements for food to meet the needs of expansion of human population. There will be an increasing and certainly believed to be proportional need to develop food products and alternative methods of insect control in order to provide this food at the desired level. Therefore, it is believed that more selective insect control measures than have been used in the past should be considered. Pest control technologies must include natural or biological enemies as well as chemical or synthetic tools.

Insect microbes have been used successfully to some extent already for control of insects. These microbes fall into three categories. Dr. Marvel mentioned them: Bacteria, virus, protozoa, and fungi. Experience has shown that if the population of these natural insect pathogens is substantially raised above that normally present, that disease is initiated among the insects and spread among these target pests, greatly reducing or removing their economic damage to crops.

Some of these microbes have potential of inducing epidemics when the insect populations are high or the concentrations of microbes become high. Thus there is a potential for microbial pathogen populations to be established that will limit and control not only present insect generations but also those into further and following seasons.

If microbes are mass produced external to their environment and released in high concentrations where target pests are found, biological control can occur. There are numerous advantages to biological control. They include a sufficient level of control at lower cost, potential for self-perpetuation after initial application, and absence of harmful effects on man, animals, wildlife, and other beneficial organisms.

Reoccurring costs, which are common with chemical applications, insect resistance, resurgence of seconday pest outbreaks, have no counterpart in biological control methods, nor does society incur long-term cost of environmental pollution, chemical residues, and effects upon applicators and nontarget species. The problem of resistance is virtually unknown in the use of microbes to control insects which affect the production of agricultural products.

At this point only one bacteria and one virus have been widely introduced into U.S. agricultural pest control programs. They are the Bacillus thuringienses or Bt, a bacteria, and a virus of the cotton boll worm/tobacco bud worm/corn ear worm complex. That is a product called Elcar produced by Sandos. However, their total sales represent only a very small portion, certainly less than 5 percent of insecticide sales in the United States.

In a recent study that our group completed entitled, "Biological Insecticides, Future Growth and Market Opportunities, 1980-1990," we surveyed hundreds of investigators and users of biological products worldwide. Their inputs concerning these types of products reveal many favorable characteristics: Preservation of beneficial insects and predators; safety to applicators and workers; ability to apply until the crop is harvested and thus exemption from residue tolerances that are required on chemicals; safety to the environment; low likelihood of insect resistance buildup, and a focused spectrum of control.

However, they have limitations, and it is in these areas of limitations that perhaps biotechnology may be able to assist in making them compete better with their chemical sisters. The limitations are seen to generally include the amount of time it takes for damage to end or insects to die because these materials act on a biological method, and they have to be ingested and regenerated internally until they cause mortality or morbidity in the insect. The care required in timing applications, they have to be timed appropriately to catch the insect at a proper stage. They have limited control of secondary pests—they are very targeted, very specific. They have a lesser level of control or percentage kill than is thought to be desired, perhaps 80 to 85 percent of the insect population is affected rather than 90 to 95 percent, as with chemicals.

Limitations exist on residuality or days of protection before reapplication, and they do have a narrow spectrum of control. It is like a rifle shot rather than a shotgun shot into a pest group.

What is clearly needed is for biological insecticides to compete better against chemicals. They need higher effectiveness, reduced cost, greater residuality, plus preserving the characteristics that are favorable

There has been in recent years increased attention to the microbial or biologial insecticide area. Much of this has come at Government levels. Perhaps more needs to be done. There has been increased attention to and awareness of this concept through the integrated pest management program; increasing concern by both public and environmental groups on the expanded usage of chemical pesticides on the environment. The increasing cost of petroleum, which is a primary raw material for chemical pesticide production, makes biological systems which can be produced on much lower cost media in a natural way more likely to be competitive.

The proposed changes in the registration requirements for biologicals will result in lower cost for meeting safety and hazard standards. The commitment that EPA has made to expedite and give priority attention to registration of biological pesticides is certainly going to help. Finally, the recent Supreme Court decision allowing the patenting of microorganisms will allow those who are interested in this area to protect their research and development investments and manufacturing capital requirements in a better way.

Improvements in the biotechnology area to microbial pesticides will come from two sources, from classical genetics—which has been discussed already to some extent by the other speakers—and molecular genetics. In classical we mean the usage of isolation, biological assay, and selection methods of naturally occurring pathogens and then using standard strain improvement methods and mutation-inducing techniques to alter and hopefully increase desired characteristics.

By molecular genetics we mean the direct manipulation of genetic material itself using DNA methods to alter the basic characteristics and responses of the organism.

It is our opinion that much improvement is yet possible using classical methods. For example, researchers at the USDA laboratories have found strains of Bt—Bacillus thuringienses—that are four to six times more active against certain target insects than those used commercially. This kind of effort must continue and should increase, as it provides a very cost effective return on research-invested dollars and these effects can be seen in a 2- to 5-year time horizon.

Logically, then, molecular genetics would begin with the most active organisms available with the objectives of further enhancement. Several bioengineering companies have announced interest in applications of these methodologies to agriculture, such as Griffen Sciences in Hartford, Conn., Genex, and Biologicals. They have indicated programs in manipulation and enhancement of microbial insecticides.

Real problems do exist and we believe will limit expansion and usage of microbial pesticides in the incorporation of bioengineering methodologies. These examples are similar to those that have been stated by other speakers. There is great need for broader, basic understanding of genetic mapping and the contributions of various segments of the genetic material to the performance and functioning of the organism. There is difficulty in the addition or altering of relevant genetic materials into attractive microbial organisms to increase functionality. The keys are not well understood.

Successful products that are affected by genetic engineering may be 5 to 10 years away but even with the current products there is basic limited knowledge of the biology of pests, of pathogens, predators, and crop timing relationships. There is inadequate understanding of the influence of the environment, moisture, sunlight, application methodology, on effectiveness and residuality, and in the understanding of basic mechanisms of infection by microbial pesticides and the influence that the environment has on efficacy.

Problems also exist in the direction that much biological research and field experience are toward scientific publications. This emphasis on scientific publications, perhaps, rather than the advancing of basic knowledge and commercial likelihood of material, is also a problem. There seems to be confusion, based on our experiences, in terms of the access to and utilization of Government-sponsored technology. It is detrimental to the exploitation and commercialization of technology. There is little enthusiasm by commercial developers to invest in technology that is in the public domain, particularly if the patenting or protection mechanisms are unclear.

Another real problem is what we see as a desparate need for more rational and clear-cut licensing and sublicensing of Government technology than is currently available. The British NRDC model is an excellent example of how this can be done. Other examples exist.

Some final problems to be overcome are the reluctance on the part of Government investigators and Government-funded researchers to move technology beyond the classical definition of research. There is considerable reluctance on the part of Government-funded investigators to move into the stage between research and development, yet there is so much basic knowledge and understanding that is missing that causes microbial systems for pest control to be—the interest in these applications to be dampened because still, in the view of the commercial enterprises, the fundamental work is yet to be done.

Finally, there seems to be a disjointed and rather random assignment of research support funds to university and private investigators. The question may seem to be more, "What would you like to do?" rather than "We have funding to support the following effort areas. What are your qualifications?"

I was very interested in the comments and the discussions earlier concerning the, I believe it is called the competitive grants program. Maybe the competitive grants program will help focus the research efforts a little more tightly. In our study, in which we looked at about 6,300 efforts in the biological area, there was relatively little correlation between the importance of the crop pest that was being studied and the amount of work that was being done.

I have to disagree a little bit with one of the conclusions that was made by Dr. Brill, in which he said that more structuring and focusing of competitive grants was detrimental, it should be loosened, money should be more general and undefined. It is our feeling that overall priorities and issue areas need to be established and administered more tightly than currently they are.

It must be recognized that markets for biological insecticides will tend to be small due to the specificness of their control. These markets will be most likely of initial interest to smaller companies, at least through the market development stage. Areas of needed action or attention that will help advance microbial biotechnology include increased screening of naturally occurring organisms with pathological control potential patterned after the Stoneville, Tex. USDA facility for Bt. This will provide a better starting point for biotechnology enhancement.

More consistent concern and support from Government agencies like the EPA, the USDA, the extension services, the State experimental stations, and regulatory groups as to the positive contribution to the environment that microbial agents make versus chemicals; clearer, better defined policies from the EPA on registration of recombinant biological pesticide products are also needed. Currently they consider this on a case-by-case basis. That is a little unsettling and discouraging to the investigators.

Clarification of the potential for patent protection of microbial pest control materials, with properties resulting from human intervention other than genetic engineering, is another area for improvement.

Finally, we see opportunities for exploiting, for example in the Northeast, the potential of using biological materials for control of community pests such as the gypsy moth. There is seemingly low awareness on the part of many of the Government officials as to the alternatives that exist to chemicals. The potential of combinations of biologicals with chemicals to reduce the impact on the environment, there has been seen to be attractive synergy in these areas.

We agree that much has been done. Microbes and microbial systems are available that will assist agriculture. The near term will be the continued enhancement, using classical genetic methods of finding and improving, isolating and refining, but the future—to provide special characteristics, to give the functionality of residuality and virulence and higher productivity in the field—may well come from biotechnologial methods.

Thank you.

[The prepared statement of Dr. Murphy follows:]

BIO ENGINEERING IN AGRICULTURAL PESTICIDES

Sheldon R. Murphy, PhD
The Performance Management Group
New Canaan, Ct., 06840

Gentlemen,

I will limit my comments to the potential for biotechnology in agricultural pesticides. This represents the area of my greatest familiarity and experience. Others no doubt will be addressing applications in plant science, agricultural waste conversion, animal science, etc.

In a landmark 5-4 decision (Diamond vs Chakrabarty June 16, 1980), the U.S. Supreme Court judged that a man-made microorganism is patentable under the current patent statutes. Furthermore, the opinion invited Congress to overrule the decision or develop statutory guidelines, if it disagreed with the courts interpretation or felt that more specific direction was desireable. The Chakrabarty opinion is considered precedent for including genetically engineered organisms within the patent law.

Previously, if a new organism has been developed after a long, expensive and difficult procedure, anyone acquiring the culture could reproduce it and benefit with little or not cost. Trade secrecy could have provided some assurance that process methodologies for optimizing yields or minimizing undesireable characteristics were kept confidential.

Therefore, this court decision is believed to have a positive impact on the attractiveness of microbial insecticides. It will provide protection outside of process technology for micro-organisms that had been designed and genetically developed from being copied, produced and used by others.

The Court decision will provide incentives for commercial development of microbiological inventions by lowering the risk and uncertainty of protection of inventions undergoing commercial development.

The Patent Office has interpreted the Court decision as permitting patents also on organisms found in nature, but with properties resulting from human intervention other than genetic engineering, such as strain improvements methods. The ramifications of this interpretation on microbial pesticides is expected to be clarified by the Patent Office actions on pending applications.

Finally, new knowledge and information revealed in the patent literature will add to the general technology of microbials. At the time of the Supreme Court decision in Dec. 1980, there were approximately 200 applications pending on microorganisms. This includes about 100 for genetically engineered microbes and about 100 cultures of strains isolated from nature.

Chemical Pesticides

In North America about 700 species of insects and other arthropods are considered pests, of these, more than one-third have been accidentally introduced from other parts of the world.

Modern technologies rely heavily on the use of chemicals to control these pests. However, the practice of near sole reliance on chemical pesticides for insect and mite control has been unsuccessful due to the attendant problems of pesticide resistance in target pests, the ever increasing need for more pesticides, with mounting costs for insect control, the reduction of natural enemies and impact to non-target species and the environment. Further, there is ample evidence that presence of broad spectrum chemicals, targeted to correct serious insect populations, have resulted in explosions of secondary insect pests, which are not well controlled by that chemical.

In total, over 200 examples of resistance are known and it is becoming nearly impossible to control some species with any chemical insecticide. Many of these compounds also have an uncertain and sometimes deleterious long-term effect on the environment.

This situation has greatly increased the development costs of traditional chemical pesticides and attempts to determine the toxicity or residual effect of these pesticides have increased the development time considerable. Also, as insects become resistant to certain chemicals, other control agents must be developed which are not derived from the same basic compound.

While the effects of pesticides on the environment and human health are difficult to assess quantitatively, the harmful effects of certain chemicals (such as leptophos, kepone and DDT) are certainly unacceptable to the American public as evidenced by the recent rulings concerning registration and use of these and related chemicals.

The losses attributable to insects as well as the increased cost of control technologies have caused concern for the future. As the food requirements increase due to human population expansion, there will be at least a proportional increase in the need for the development of alternative insect control technologies. There is also need for more selective insect control measures than those used in the past. Pest control technologies _must_ include _natural_ or _biological_ enemies, as well as, synthetic tools.

Microbial Pesticides

Insect microbial control activities fall into four categories: Entomopathic microorganisms (bacteria, viruses, protozoa, fungi). Experience has shown that if the population of some natural insect pathogens is substantially raised above that normally present, diseases are initiated and spread among the target pests, greatly reducing or removing their economic crop damage potential. Thus, some microbes have the potential of inducing these epidemics (epizootics), when the insect population or the concentration of microbes become high. There are also indications that when some microbial pathogen populations are established, their potential to limit or control carries into subsequent insect generations and even the following season.

Therefore, if microbes are mass produced, external to the envirnoment, and released at high concentrations when target pests are found, biological control can occur.

Advantages of biological control are numerous. They often include a sufficient level of control at lower cost, potential for some self-perpetuation of control following the initial application effort and reduction or absence of harmful effects on man, animals, wildlife and on other beneficial organisms.

Further the recurring costs of chemical applications, insect resistance, and resurgence and secondary pest outbreaks have no counterpart in biological control nor does society incur the long term costs of environmental pollution, chemical residues and effects on applicators and non-target species. Finally, the development of resistance to introduced biological agents, with the consequence of imperiling the whole program, is virtually unknown.

At this point only one bacteria and one virus have been widely introduced into U.S. agricultural pest control programs. They are Bacillus thuringienses or Bt (a bacteria) and the nuclear polyhedrosis virus from the cotton boll worm/tobacco bud worm/corn earworm complex. Their total sales represent only a small portion of the insecticide sales in the U.S. (less than 5%).

A recent Study our Group completed, "Biological Insecticides, Future Growth and Market Opportunities, 1980-1990", surveyed hundreds of investigators and users of microbial products worldwide. Their inputs reveal that these products have many favorable characteristics:

- preservation of beneficial insects and natural insect predators
- safety to applicators and workers
- ability to apply to the crop until harvest (exemption from residue tolerances)
- safety to the environment
- low likelihood of insect resistance buildup
- focused spectrum of control

However some microbial insecticide limitations are some seen as critical by the farmer/grower. They include :

- the amount of time it takes for damage to end or insect to die
- care required in timing applications
- limited control of secondary pests
- level of control or percentage kill
- residuality or days of protection before reapplication
- narrow spectrum or width of insect control

What is clearly needed for biological insecticides to compete better against chemicals (which have a high impact on the envirnonment) is higher effectiveness, reduced cost and greater residuality, while preserving all of the unique, desireable features mentioned earlier.

Several major factors have, during the past few years, contributed to the increased attention to microbial insecticides for crop protection:

- increased attention to and public awareness of the concept of a coordinated, integrated appraoch to the management of pests (Integrated Pest Management - IPM)
- increasing concern by the general public and environmental groups concerning the risks of expanded chemical pesticide usage on the environment, the econlogical system and on non-target species.
- the increasing cost of petroleum, which is a primary raw material for much chemical pesticide production
- proposed changes in the registration requirements, with resulting lower costs for meeting safety and hazard standards for biological products
- commitment by the EPA to expedite and give priority attention to registration applications involving biological pesticides
- recent Supreme Court decisions, allowing the patenting of microorganisms

However, important improvements are needed. They include:

- increased microbe virulence
- higher production of toxins
- higher increased production rate
- more environmental stability/adaptability
- ability to use lower cost growth media
- capability of controlling a wider spectrum of target insects.

Two methods have potential for reaching these needed improvements:

- classical genetics, including usage of isolation, bioassay and selection of natural occuring pathogens and then using strain improvement methods and mutation inducing techniques to alter and hopefully increase desired characteristics.
- molecular genetics which involve direct manipulation of the genetic material itself using DNA methods to alter basic characteristics and responses of the organisms.

It is our opinion that much improvement is yet possible using the classical methods. Researchers at the USDA laboratories have found strains of Bacillus thuringienses which are 4-6 times more active than those commercially used against certain target insects. This important effort must continue and should increase, as it provides the best, most cost effective return on the invested research dollar, in the near term, 2-5 year time horizon.

Logically, molecular genetics whould begin with the most active organism available, with objectives of further enhancement. Several bioengineering companies have announced interest in application of methodologies to agriculture. Some such as Griffen Sciences, in Hartford, Ct., Genex and Biologicals have indicated specific interest in manipulation and enhancement of microbial pesticides.

Real problems are seen to still exist that will limit expansion of usage of microbial pesticides and incorporation of bioengineering methodologies:

- the need for broader basic understanding of the genetic mapping or contribution of various segments of the genetic material to the performance and functioning of the organism.
- the difficulty of the addition of altering of relevant genetic material into attractive microbial organisms to increase functionality. Successful products may well be 5-7 years away.
- limited basic knowledge of biology of pests, pathogens/ predator and crop timing relationships.
- understanding of the influence of moisture, sunlight and application methodology on field effectiveness and residuality.
- understanding of the basic mechanisms of infections by microbial pesticides and the influence of environmental factors on their efficacy.
- the direction of much biological research and field experiences are toward scientific publications, rather than advancing the basic knowledge and commercial likelihood of the material.

- confusion in access to and utilization of government sponsored technology. This is detrimental to the exploitation and commercialization of the technology. There is little or no enthusiasm by commercial developers to invest in technology that is in the public domain.
- desperate need for more rational and clearcut licensing and sublicensing of government technology, than is currently available. The British NRDC model is excellent.

- reluctance on the part of government investigators and funded government funded researchers to move technology beyond the classical definition of "research". So much basic knowledge and understanding is missing in the application of microbial systems to pest control that commercial interests are dampened by the amount of fundamental work that still remains to be done.
- seemingly disjointed and random assignment of research support funds to universities and private investigators. The questions seem to be more "what would you like to do" rather than " we have funding to support the following effort areas - what are your qualifications". In our Study we compared the incidence of research investigations (based on literature citations) against the relevant importance of the crop pests under investigation. There was low correlation between the two.

It must be recognized that markets for biological pesticides will tend to be small, due to their specificness of control. These markets will more likely be of initial interest to smaller companies, certainly in the market developemnt stage. Areas of needed improvement which will help advance this microbial-bio technology include:

- increased screening of natually occuring organisms with pathological control potential, tailored after the Stoneville, Texas - US Department of Agriculture facility for Bt.
- More consistent concern and support from government agencies, ie, EPA, USDA, extension services, state regulatory groups as to the positive contribution to the environment that microbial control agents provide versus chemicals.
- clearer, better defined policy from the EPA on registration of recombinant microbial pesticide products. Their case-by-case rationale is discouraging.
- clarification of the potential for patent protection of microbial pest control materials, with properties resulting from human intervention other than genetic engineering.

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CONSULTING TO TECHNICAL AND COMMERCIAL DEVELOPMENT MANAGEMENT - providing business and professional expertise to improve product line performance through the application of innovative program introduction strategies and management.

QUALIFICATIONS

Over fourteen years of demonstrated leadership, consulting and problem solving via highly creative team efforts for three major corporations, operating multinationally. Skills include technical consulting, team reorganizing and building, program direction, market strategy analysis and development, quality improvement, research planning and program support, instrumentation development and process automation, computer science and information services.

Made money for corporations by being a catalyst for positive and constructive growth and change. Developed strong teams which led the way towards new product introduction, process improvements, laboratory and process automation, innovative problem solving, introduction of modern management methods, solution to sophisticated marketing situations and direction to management decision making.

PhD in chemical engineering and mathematics from the University of Utah - 1965. Worked 3 years in Brazil. Fluent in Portuguese and conversational in Spanish. Age 44.

EXPERIENCE RECORD

Director Research and Development, FMC Agricultural Chemical Group
Responsible for all phases of compound synthesis, evaluation, field testing, product EPA registrations, environmental chemistry, automated screening, process development programs. Managed 300 people and $15MM budget at three locations.

Manager - New Product Ventures, FMC Agricultural Chemical Division
Developed the research, marketing, manufacturing and raw material procurement strategies for marketing of new technical product: POUNCE. Formed task force to accelerate commercialization. Before registration sales exceeded $40MM.

Assistant Director of Technology, FMC Chemical Group
Managed 175 person technical staff departments in two locations. Staffed professional specialists in lab and introduced process automation and statistical, computer science techniques. Process automation efforts improved quality and throughput in two chemical process locations, with annualized savings at $1.7MM. Audited overall Chemical Group technical programs.

Director, Johnson & Johnson Corporate Management and Technical Consulting Services
Established J&J's first in-house consulting function: hired and trained professional staff specializing in management science and computer operations, market research and computer science services to divisions. Pioneered risk analysis techniques; identified new business opportunities; solved manufacturing problems through automation; created marketing strategies to introduce new products.

Director, PepsiCo Corporate Engineering and Quality Assurance
Upgraded quality assurance, laboratory services and engineering programs in newly formed Corporate technical division. Directed programs for reorganizing laboratories, more relevant specifications and effective engineering/processing efforts. Responsible for twelve laboratories (nine international), developed computer automation and statistical analyses/information handling systems.

Mr. **Volkmer** [acting chairman]. Thank you, Dr. Murphy. I have a few questions and then staff may. The chairman had to leave to go to another committee meeting, and I am supposed to be at another one right now, too, so when he comes back I am going to have to leave.

However, I would like to ask Dr. Kaufman if he would comment on the stability of induced traits through regeneration.

Dr. **Kaufman**. Do you mean seed transmission?

Mr. **Volkmer**. Yes.

Dr. **Kaufman**. It has been a mixed bag. In some cases if it is a single dominant gene the transmission works very well. In other cases it can get more difficult. Some of the changes induced in tissue culture seem to involve chromosomal aberration, and those are not well transmitted sexually, so it is a case-by-case study and that just means more research to collect more variants that have the desired traits.

Mr. **Volkmer**. There is some discussion with a previous panel basically made up of university personnel, comments made as to the need for additional researchers, at least studies at the university level on—I guess it has been called plant biotechnology. Would anyone on the panel like to comment on that?

Dr. **Kaufman**. I think I would endorse it for sure. I would not call it plant biotechnology as much as I would basic research into plant biochemistry, physiology, molecular genetics, things of that nature.

Mr. **Volkmer**. Fine.

Dr. **Kaufman**. We have an amazing void in our knowledge base on plants, and we are not going to be able to overnight fill that void with quality people. We have to begin to woo people away from the health sciences and the medical sciences and make them aware that plants are an interesting and challening area. It is going to take us 10 years, easily, to build up the kind of plant expertise that we currently enjoy in the animal fields.

Mr. **Volkmer**. Anyone else wish to comment?

Dr. **Murphy**. A similar problem exists in the entomology area. There is a lot of basic knowledge that is just not well understood with regard to how insects operate, hox they damage, how they generate, how they carry over from season to season. That understanding is necessary in order to interact and effect their systems either with chemical or biological methods.

Mr. **Volkmer**. All right now, where is the problem as you see it—I will go back to your comment last, Dr. Murphy—on the impetus needed, in other words, or the emphasis needed in order to have more research done in these areas. Is it at the university level?

Dr. **Murphy**. Certainly. I am not sure how quickly the concept of supply and demand operates at the university level. There must be a 2- or 3-year lag between identified areas of high interest and support to the creation of qualified students or graduate students but it is our opinion that there is need for basic understanding. In these emerging areas such as biological pesticides, this basic understanding will have to come from Government laboratories and universities because the businesses are so small, they cannot develop them internally.

The commercialization of concepts and knowledge that has emerged from these sources can occur and is being attempted. The success is still very limited but the more experience that is gained in the field with regard to failures, the more identified the needs for basic knowledge and familiarity with the systems has become.

Mr. VOLKMER. Anyone else? Dr. Marvel?

Dr. MARVEL. I think that one of the things that the subcommittee might consider is some of the remarks that were made earlier and have been made now, that the field of agriculture, for one thing, is an extraordinarily broad field and there are not many people available to recruit who have broad understanding of plant systems in either a physiological or a biochemical sense. The training of those people probably has to come from fundamental places and I think one thing that ought to be kept in mind is that the objective of this effort is to deliver a viable, useful product to farmers. In order to do that, I think you have to consider two things. One of them is, I think you probably need to consider ways to bring scientists from multiple disciplines together and they probably need some practical guidance as to people who are familiar with agriculture.

I think what has made the agricultural system of the United States very strong is that there has probably been better cooperation in agriculture, from industry and from the Government and from academia, than probably in any other single field. Because of that cooperation—and one might take advantage of it—but when the cooperative and extension people in the USDA go to the farmer, they are familiar with these methods. I would hate to see us lose that in whatever funding mechanism we used in order to bring more and better basic scientists into the field of agriculture, so I would say that some granting mechanism needs to be considered and where it should be in terms of how you would coordinate that to be sure you do give incentives to biochemists, molecular biologists, physicists, mathematicians, computer people, chemists. How do you make them jell to where the problems really are?

Mr. VOLKMER. Thank you very much. I will then turn over the chair to the gentleman from Tennessee.

Mr. GORE. Thank you. Thank you very much.

I apologize for having to leave momentarily. We had a witness from Tennessee downstairs on why there are no gasoline signs in many of our gas stations now, and that is a subject of more than passing interest to my constitutents.

Dr. MURPHY. Mr. Chairman, may I add to Dr. Marvel's comment?

Mr. GORE. Please do.

Dr. MURPHY. The point of multidiscipline approaches to solve some of the technology transition problems is very relevant. It is our view that the advances in microbiology far exceed the advances in entomology and agricultural crop practice understanding, and therefore from a microbiological point of view, there are materials that are known that can be used, that can be enhanced, but how to apply them, when to apply them, how to preserve and protect them in the environment of the agricultural crop production system is not known. Therefore, the interactions and the synergy that needs to come in order to have a successful impact on agriculture is still missing to a large extent.

Mr. **Gore**. Very good.

Dr. Marvel, you state in your testimony that the basic gap in knowledge in biotechnological applications to agriculture is a troubling one and you say, "We know very little about how genes are organized, controlled, or expressed in plant cells." That or a similar statement has been made by other witnesses today, and it leads to a basic question that I would like to direct to the whole panel.

Do you think that particular aspects of this technology have been oversold or hyped, such as nitrogen fixation, and that these large gaps are being ignored?

Dr. **Marvel**. Well, I think that the need for fundamental knowledge is going to be extremely important to whether any efforts on nitrogen fixation can be brought to fruition. I think the most ardent exponents of the miracles of biotechnology to the most conservative, I do not necessarily think there is a big debate about what it could do. There may be a fairly significant difference of opinion about the time frame that it may come about but I certainly believe that there is a great abundance of fundamental knowledge. Maybe to make it more graphic, it is not just cellular control of genes in plants but the physiology of a plant, and I think Dr. Frey referred to this in some sense.

We do not know the events that lead to why a corn plant yields certain ways and does certain things, just the gross aspects, not the molecular level aspects. It is pretty hard to focus on those molecular level aspects with great precision if you do not understand the physiological parameters, so we are even further back, maybe, than I indicated.

Mr. **Gore**. Dr. Kaufman?

Dr. **Kaufman**. I would just reiterate what I said earlier. I think that there are enormous gaps in how plants are put together, in how enzyme systems tie together to effect the reactions that ultimately result in yield. It is going to take some time to sort those out and it is going to take some time to attract people to get involved with sorting out those problems.

Perhaps plants have been a victim of public relations problems, too. Plant biochemistry is exceedingly difficult and it is complicated by two factors: One, plants produce phenolics, and the second, plants have cell walls. Other living systems do not have these complications and it makes their biochemical investigations considerable more straightforward. Therefore, for a biochemist originally looking for problems, plants were not attractive.

However, I think as more and more hype is given to the food crunch that we are going to be in in the next 25 to 35 years, scientists will be more and more attracted to this difficult area. I think with the provision of money from both Government and private industry we should begin to get a handle on these kinds of problems.

Mr. **Gore**. Dr. Frey?

Dr. **Frey**. Yes, I agree with what has been said. If there is a problem with hype, I think a lot of the problem is a lack of people who can translate or a lack of data that will allow us to translate from, let's say, the molecular level, the single cell level, the microbe level, whatever the case may be, through the whole plant or agronomic system. I mean, we have to go from the cell to a whole plant and then a whole community of plants across a wide range of environmental and climatological stresses or whatever the case may be.

It is hard to project effects on crop yield from a 15-percent increase back here at this molecular level. If I am very specialized in my research at the molecular level, (I know a deal about it, but) how do I translate from that level all the way to the field crop? We do not have many translators. There are not many people that are working actively in that area, trying to talk to breeders on the one hand but still sophisticated enough in their basic knowledge to talk with the microbiologist or the plant biochemist or whatever, to tie this all together.

Therefore, I think if we sometimes overstate a case of what will be achieved, it is just a matter of not really knowing how to translate the data before me into an applied agronomic system.

Mr. GORE. Dr. Murphy?

Dr. MURPHY. I suspect, Mr. Chairman, that you would like suggestions from us as to what could be done because there seems to be a common theme that there are large gaps in our understanding that will allow advanced technology, such as the biogenetic engineering to assist agriculture. These gaps do exist. During the 1960's one of the programs that helped give the United States some advanced steps in the space area was not only the NASA program but as applied at a university level, the National Defense Education Act. Maybe we have need for a national agricultural education act of some sort to sponsor focused activities and programs that will assist people in gaining graduate-level knowledge and understanding in multidisciplines to assist in the transfer problem, because it is hard for a single individual to gain much of the information through the conventional programs to be able to fill the voids of knowledge that exist.

Companies seem to be able to do this better than universities because they by nature are multidisciplined. Therefore, if this is to be stimulated there need to be incentives for companies to do this, either easing regulations or facilitating relationships or licensing methods or some realistic way of helping those problems and those information bases that exist in bits and pieces to be assembled so that consistent efforts can be undertaken.

Mr. GORE. Well, that is an interesting proposal and one that this subcommittee may well pursue in recommendations and a report after this hearing and the next one.

Let me ask you, Dr. Marvel, and I would appreciate a brief response because I have a couple of other questions and then we will try to finish this panel before the vote on the floor: Is it true that genetically engineered "Roundup" resistance in alfalfa, resistance to this chemical, could have positive effects on the effort to control soil erosion?

Dr. MARVEL. I think that is more of a farming practice mechanism than the "Roundup." It could have some in the context that you use no till. You would not have to use "Roundup" on bare ground. You could use it with something planted in it, which would help erosion but I do not think the effect would be very dramatic in that sense.

Mr. GORE. Is Monsanto undertaking any research concerning possible genetic engineering applications in animals?

Dr. **Marvel**. Yes, they are intimately involved in bovine growth hormone work right now.

Mr. **Gore**. The subcommittee is going to have a followup hearing to this one focusing on animal biotechnology. Do you feel comfortable answering questions about that?

Dr. **Marvel**. I could do a little bit but I think it would be better if we arrange for someone who is actually working in that area to speak to you.

Mr. **Gore**. OK, we will do that. However, just briefly, looking way down the road, is there some point at which you envision the possibility to literally clone livestock?

Dr. **Marvel**. I think that possibility would exist. I am not sure ethically how either I or anybody else is going to feel about that kind of thing in either human or animal health but I think that possbility you have to conceive as theoretically possible.

Mr. **Gore**. Well, we are going to explore that in another hearing.

Dr. Frey, there seems to be some difference in the level of impact you and Dr. Kaufman predict for your respective industries. Is this a function of differing viewpoints as to what is scientifically possible or is it merely an accountant's call?

Dr. **Frey**. First of all—I think one of the biggest things on my views in—Pioneer assesses biotechnology advances as more long term and maybe with less potential economic return because we are only looking at the crop. That is not nearly as big a market potential—we have a lot narrower focus—as a company the size of Monsanto foresees. They can span the range from the crop all the way to animal growth hormones, to some process that would enhance their chemical operations. Monsanto could use a biological product, an enzyme or a culture for fermentation to do something that they are now using expensive fossil fuels on, thus they can afford to expend a lot more dollars because they have a larger potential payoff than Pioneer, who only sells seed of six agronomic crops. The markets are just different.

Mr. **Gore**. I see. Therefore, you do not really differ in your view of what is scientifically possible and what is likely?

Dr. **Frey**. Not at all, no. I just think the plant system is longer term and it is going to take a time. I think we still do not even know the size of the market for biotechnology improvements in plants because we do not know what is possible. So Pioneer tends to have a lower level of funding activity for biotechnology than other people.

Mr. **Gore**. All right. Very good.

We have another vote on the floor, this time on the congressional tax deduction, and I do not want to miss that vote.

We have one final witness, and with the indulgence of our final witness, Dr. Still, I would like to come back at 1:50 and finish up. That will give people a chance to get a quick bite to eat downstairs if you would care to and we will come back in after a brief recess at 1:30.

Let me thank all of the witnesses on this panel for a very fine, excellent contribution to our understanding in this area. We deeply appreciate it. Thank you very much.

AFTERNOON SESSION

Mr. GORE. The subcommittee will come back into session. We will hear from our final witness, Dr. Gerald Still, with the Agricultural Research Service, USDA in Beltsville.

Dr. Still, welcome. Thank you for your patience in sitting through this morning's testimony. We appreciate the fine work that you do and we look forward to hearing from you.

STATEMENT OF DR. GERALD G. STILL, NATIONAL RESEARCH PROGRAM LEADER FOR PLANT BIOCHEMISTRY AND PHYSIOLOGY, AGRICULTURAL RESEARCH SERVICE, U.S. DEPARTMENT OF AGRICULTURE

Dr. STILL. Mr. Gore, it is a pleasure to be here and I think it is only appropriate that we have this little break to partake in the products of agriculture. Fortunately, in this country most folks subscribe three times a day, some more than others. I think rather than read this whole text that you have before you I would rather skip through it——

Mr. GORE. That is fine.

Dr. STILL [continuing]. And point out a few highlights.

Mr. GORE. Without objection, the entire text will be in the record in full.

Dr. STILL. Very well, sir.

I would like to point out to begin with that even though I represent the Agricultural Research Service, that I like to look upon as the people's agricultural research institution, I would like to address my comments to your committee on a broader base and that is the agricultural research institution, of which there are several parts, all of which have been at my position at this table in this morning's session: That is, the public on the Federal side, which will be the cooperative State research service, of which the competitive grants is a part; the Agricultural Research Service, which is the full-time, long-term, high-risk commitment to research in the field of agriculture, the broad breadth; our State contributors through the State agriculture experiment stations; and of course the private sector that is so much a part of this institution.

Now we meet today to discuss the impact of genetic engineering, in this vital sector of our economy we call agriculture. The agriculture sector may one day be one of the largest beneficiaries of genetic engineering research being conducted today. Genetic engineering, however, is not going to be a panacea for all of the problems facing agriculture.

Simply stated, genetic engineering or molecular biology and gene splicing, as you wish to call it, is a new tool. It is a new tool for transferring and expressing genetic information between and within genera and species of plants, animals, and microbes. It is as important a tool as any of the other tools we have in our laboratory but let's keep it in perspective.

Genetic engineering is a technology for which there are great expectations, as has been witnessed this morning. It is also a technology in which we have little experience. Its promises of new cures for diseases and increasing productivity in plants and animals are many but the realization of these promises to date are few.

Now, Mr. Chairman, I would like to divide the field of genetic engineering into two parts because it is rapidly moving, and it became clear this morning that these two parts were becoming confused. I would like to divide it on the basis of the complexity of the genes and the background of those genes in which these products are going to be expressed.

First, what I call the fermentation delivery system is based on a very simple genetic background: It is fungi, bacteria, and we know a great deal about this, Mr. Gore. It is a small, simple gene, and because of the very large investment that has been made here and abroad in the last number of years it is well mapped. We know how to apply the technology and it will plug right into the existing fermentation industry. Whether it be foot-and-mouth disease as I described in the text, that was done in the Agricultural Research Service and with Genentech Corp., or whether it is done with one of the large pharmaceutical companies, there is a literature that exists that will allow us to apply that technology today. When Dr. Brill made his comment, I would concur: That part of the genetic engineering story, the fermentation delivery system, is one that is purchased and has the leverage to be applied.

I would suggest that because of the long commitment at the Agricultural Research Service and now with the cooperation with Genentech Corp., that the world will have a vaccine for the 11 major strains of foot-and-mouth disease, and I need not counsel you on how important that is.

Now if we move from that scenario of the simple gene and the simple background and address the other segment of the story that you heard for the most part discussed this morning, that is the germplasm delivery system and its application to agriculture. Now in this system we are talking about the complex background of plant and animal genes and the expression of those genes.

As mentioned earlier, there are some difficulties associated with this approach such as the complexity of the plant and animal genes themselves and the lack of a backlog of understanding and information on how these genes are expressed. The germplasm delivery system is nevertheless a concept in which private industry is investing and the payoff is projected to be in the billions of dollars.

Major emphasis is being directed toward plant improvement by both public and private research groups, as you have heard this morning, plants that will increase their efficiency and production by introducing new genetic traits, more effective abilities to adjust to environment, to resist pests, and to produce products of greater quality. Let me just simply add, on this business of efficiency, I would say it is quite true that our yields of corn are on a positive slope. That is bushels per acre. However, I should also bring to the committee's attention that if we are going to meet the world needs as projected in the future, we had better address the concept of efficiency, that is, the amount of nitrogen, water, soil, and what have you to maintain and sustain that yield.

Now it is my understanding that the only agronomic system that indeed has increased its efficiency is in poultry and swine.

The future of genetic engineering research and the germplasm delivery system will rely on cooperation between State and Federal scientists and those employed in the private companies, and this is one of my primary concerns. Such cooperation has existed in the past. However, the state of our science has changed and there are new problems to be solved, questions such as where should society invest its limited resources and in which sector of society should this science be accomplished.

Now there are three parts to this problem which I would like to examine with you today: The human resources, the basic knowledge, and the performing institutions. You will recognize that these are not new concepts. You have heard about them also this morning. Let's reinforce them.

Anyone involved with genetic engineering recognizes that there is a human resource crisis. There are individuals in this room who have had a productive career in public research and teaching and are now moving into private research. Mr. Chairman, if this trend continues, where are we going to develop the talent that will be needed in the coming years?

Another dimension of this human resource problem is brought about because of the relatively small community of experts that are capable of working in this area of molecular biology. This is not only causing competition for this resource but it is resulting in a series of public and private cooperations. These cooperations, however, are raising institutional questions that must be considered.

I would like to quote a clipping from Dr. J. P. Kendrick, Jr., the University of California's vice president for agriculture, who I think states it very succinctly:

> Storm clouds are gathering, clouds that could coalesce to bring a storm of public criticism down upon our university community unless we plan carefully to protect the integrity of free inquiry and unrestricted exchanges of scientific information.

Free inquiry and unrestricted exchanges of scientific information—

> Questions of ownership of the discoveries arising out of publicly sponsored research programs must be addressed and resolved. The role that patents play in providing incentives for capital investment to develop ideas and discoveries into useful products must be carefully defined and vested interests in such development must be attained. Further, there is a need to determine who the parties of vested interest are before formulating policies to guide the relationships between public and private entities.

These are the kinds of issues that must be considered and resolved as institutions address the short-term and the long-term problems of the human resource base in this rapidly moving field of genetic engineering.

Now the second issue to consider is the basic knowledge. We would not be gathered here today to discuss genetic engineering in agriculture if the United States and Europe had not invested heavily in microbial genetics and enzymology, the very stepping stones that make up the field that we are discussing. I do not believe that we have invested heavily enough in basic knowledge in the area of the germplasm delivery system.

For example, as we have heard from our colleague from Pioneer Seed this morning, we do not know enough about the physiological genetics, the cytogenetics, or the inheritance factors in plants and animals. The development of gene transfer vehicles is important but even more important is the knowledge of the structure, the organization, and the mechanism of expression of the myriad of agriculturally important genes.

For example, plant breeders are seldom interested in a single gene addition or substitution, but rather in the interactions among scores or hundreds of genes in which the total make up the commercial acceptance of their product, which is indeed the seeds that go into the hands of our farmers and ranchers. As you know, this is part of an extensive community of agricultural research, and those historic lines are in place but, as stated this morning, it is my assessment that genetic engineering will augment those institutions and not replace them.

OK. It is the complexity of the genetic material that separates the fermentation and the germplasm delivery systems, and it is this genetic complexity which is the knowledge gap in the data base of plants and animals that must be filled before the expectations of the germplasm delivery system may be realized. These are areas of research that we have to address as enthusiastically as we do the more popular areas known as genetic engineering.

Now perhaps what is needed are some mechanisms that will bring together the existing expertise of several disciplines of research and education, a mechanism that would bring together agriculturalists and chemists, the schools of medicine and microbiology, to study and increase our data base in crop plants and farm animals. Mr. Chairman, I am convinced that this generation has the responsibility to establish a knowledge base which future generations may draw on.

The third consideration are those research institutions that are best able to conduct genetic engineering research, and I think we will find that there are roles that can be played by both public and private institutions. I believe the public will have to invest in the long term, high risk, expensive basic research that drives science into new fields. The private sector will conduct research to further develop those concepts and technologies and then commercialize their findings.

I believe that without a combination of Government backed basic research and industry backed research to produce the product development and commercialization, many products such as the foot-and-mouth disease vaccine will not be introduced into the marketplace quickly or at a reasonable cost.

As stated at the outset, there are many expectations for genetic engineering results and little experience on which to base them. The expectations can be met if this Nation invests in research and therefore provides experience. Agriculture needs genetic engineering. This new tool will be applied using both the fermentation and the germplasm delivery systems. The expectations will be met if we invest and provide the experience with the new tools.

I thank you, sir.

Mr. GORE. Thank you very much.

You seem to agree with the other witnesses when you say, "I do not believe we have invested heavily enough in basic knowledge in the area of the germplasm delivery system," but the budget for this specific technology continues to be cut. Am I inaccurate on that?

Dr. STILL. No, sir, you are accurate on that.

Mr. GORE. Well, I can only assume, then, that these decisions are ones that you either do not support or are constrained to give lip service to by reason of your institutional position.

Dr. STILL. That is correct, sir.

Mr. GORE. OK. All right.

Now testimony this morning indicated that only 1 percent of the total agricultural research budget is going into this area. If we in the Congress can figure out some way to free up some resources to increase the level of attention paid to this area, I take it that you would recommend that we do so?

Dr. STILL. Yes, sir.

Mr. GORE. Well, that is certainly forthright testimony. I wish all the witnesses we had from the executive branch would lay it on the line like that.

You focused on the Agriculture Research Service's role in the development of a genetically engineered hoof- and-mouth vaccine. What other efforts is the USDA making to apply genetic engineering techniques to animals?

Dr. STILL. In the animal field, Mr. Chairman, I can only speak with authority from the viewpoint of the Agricultural Research Service. There are a great number of economically important animal diseases that will undoubtedly respond to a scenario similar to the one used for foot-and-mouth disease. In other words, instead of using a whole attenuated virus, an engineered bacteria that will produce a segment of the protein coat that can be used as a vaccine that will eliminate the probability of spreading that disease, particularly with the exotic diseases.

In the Agricultural Research Service, Plum Island Laboratories are working in this area, as are the laboratories in Denver, Colo. There are a number of diseases. If you wish, we would be happy to supply in written testimony for the record.

Mr. GORE. Based on the OTA report, just how important does USDA consider research?

Dr. STILL. Well, sir, the Agricultural Research Service is, indeed, a research institution, totally, and our commitment is, yes, to meeting the needs of those farmers and ranchers who measure their success in red and black ink but it is incumbent upon us to provide the means, such that the technology is available to those farmers and ranchers. Now whether that is seeds of better genetic traits or whether it is agronomic practices, or whether indeed it is development of materials of agricultural origin that find their way into the marketplace, similar to the shirt you are wearing, that comes out of our regional laboratory in New Orleans.

Mr. GORE. Well, I understand that, and to some degree you were very candid in responding to my first question in this area. However, for the record let me just make this point a little sharper: The Department of Defense is spending 30 times as much, not just in real terms—many thousands of times as much in real terms—but as a percentage of its total budget it has a 30-times-greater percentage of its total budget on research than does USDA. Your 1.5 percent of your budget spent on research compares to 16 percent at Energy, 12 percent at Health, Education, and Welfare, and as I mentioned, 45 percent in DOD. That certainly seems like a disturbing disparity. You stated your own view but I will give you an opportunity to make any other comment that you want.

Dr. STILL. Of course, the percentages you are citing, sir, are the entire USDA budget, I would guess.

Mr. Gore. Yes.

Dr. Still. Let me respond, sir, as a scientist. As the testimony states, as a scientist I do not feel that this Nation is investing appropriately in this fundamental area. In order to meet the needs of the future, if we are really going to have to double the agricultural output, we had better start investing in fundamental knowledge.

Mr. Gore. Well, I certainly agree with that, and I think I speak for this subcommittee. I think your statement is an extremely helpful one.

Do you agree with Dr. Wood's earlier assessment that the AES system should be expanded for training?

Dr. Still. Sir, we have a long history of cooperation between the Agriculture Experiment Stations and the Agricultural Research Service. Both of these institutions must be maintained healthy.

Now the training in our agricultural universities is a key issue. If I was asked, and I presume I am, I would recommend that the committee consider something similar to the training grants that the National Institutes of Health used—post-Sputnik. I think we need to tie the traditional segments of the agricultural universities with the nontraditional departments, botany, medicine, physics, in order to meet the needs that we are discussing today.

Mr. Gore. Would you be willing to respond to additional questions in writing for the record?

Dr. Still. Yes, sir.

Mr. Gore. We have another vote on the floor and there is going to be a markup of legislation in this room by the time the vote is over with, so we may submit additional questions to you for the record.

I want to compliment you on your excellent statement and your candor in responding to the questions, because this is an incredibly important venture for our country to make, and we are not doing it well now. We need to step up the effort. That is clear to every thoughtful observer in the area, and I am gratified that it is also clear to someone of your stature within the USDA.

As one Member of Congress, I am certainly going to do what I can to try to translate your testimony and the testimony of the other witnesses in this hearing to my colleagues in the form of an additional commitment by the country to this very needed investment.

With that, thank you very much.

Thanks to all of the witnesses who have helped us in this hearing today. We will have another hearing next month on the animal biotechnology and I am sure we will hear from you or some of your colleagues in that hearing.

With that, the hearing is adjourned. Thank you very much.

[Whereupon, at 1:55 p.m., the subcommittee recessed, to reconvene at the call of the Chair.]

POTENTIAL APPLICATION OF RECOMBINANT DNA AND GENETICS ON AGRICULTURAL SCIENCES

WEDNESDAY, JULY 28, 1982

House of Representatives,
Committee on Science and Technology,
Subcommittee on Investigations and Oversight,
Washington, D.C.

The subcommittee met, pursuant to call, at 10:30 a.m., in room 2325, Rayburn House Office Building, Hon. Albert Gore, Jr. (chairman of the subcommittee) presiding.

Mr. Gore. The subcommittee will come to order. I would like to welcome our witnesses and guests.

One of the primary efforts of this subcommittee during this Congress has been to identify some of the most important technologies of the future and assess their potential impacts.

We have examined such technological developments as microprocessing and robotics, and a great deal of our attention has been focused upon the potential implications of genetics and recombinant DNA.

Our examination of the potential for genetics has been a diverse one. We have looked at many implications: ethical, legal, and economic, as well as the clear interest in the science itself.

Most recently, we have examined the potential in agriculture, partly because so many experts have stated that this is the first sector in which we can expect the genetic revolution to take place.

Last month we began by investigating possibilities for the plant sciences and learned that by the end of the century the way our crops are grown will not be at all the same.

Today our effort will be to conduct a similar inquiry into animal breeding.

As we all know, it will be some time before we can actually clone a prize-winning Angus. A bovine cell is at least as complicated as that of a human. However, even without cloning, the advances in embryo and genetic manipulation could still mean a revolution in livestock breeding as we know it.

Since we are developing the ability to pick and choose the traits we want in our stock, much of the guesswork could be rapidly taken out of the animal breeding business. This is certainly a trend that we ought to be familiar with.

Specifically, we are interested in the science involved in embryo transfer and about the burgeoning potential for getting desired traits by transferring genes.

An equally important consideration to what is possible is the question of when these developments are likely to be widely available.

Third, we are interested in the potential impacts on the livestock industry that we might expect from these technological advances.

Lastly, we would like to hear from those involved in this field of scientific inquiry, as to what is the appropriate role of the Federal Government in this research. This is a most important consideration in that if a revolution is on, it is my hope that these technologies could be widely disseminated.

Therefore, it is important that we congratulate the USDA for apparently being at the forefront of these developments, as it is not always the case that the Government is extolled for being on top of things.

The Federal investment in this arena has been, in large part, to insure the viability of U.S. agriculture, large and small alike, and I encourage the USDA to keep up their efforts.

[The opening statement of Mr. Walker follows:]

OPENING REMARKS OF REPRESENTATIVE ROBERT S. WALKER

Mr. Chairman, I represent one of the finest farming areas in America and our hearing this morning is of great interest to me, and to my constituents.

As I was reading the materials getting ready for our hearing I was astonished to learn of some of the things that are now being accomplished in this area.

Some of the techniques now being developed seem to be almost beyond belief, but the frontiers of science are expanding so rapidly that what was science fiction only a few short years ago is not commonplace.

I am pleased to see that the Department of Agriculture is deeply involved in this new technology. At least in my part of the country the small family farm is still the norm. We don't see too much of the agribusiness which has become prevalent in other parts of the country.

But it is essential that we remember that the small farmer can only survive today for as long as he can get an even break in getting and utilizing new technologies. With the Department of Agriculture doing much of the research in these technologies we can expect them to remain in the public domain and be available to the small farmer at a reasonable cost.

Some of the things we will be hearing about today are truly astonishing. One is the technique of giving a cow hormones so that she will super-ovulate, and then the fertilized eggs can be implanted in surrogate mothers. It is obvious that this technique can, and undoubtedly will, lead to tremendous improvements in the grade of animals available to the average farmer.

Some estimates are that milk production can be quadrupled, and that the ratio of red meat to fat in cattle can be considerably improved.

These are important advancements, and they will have a significant impact on our agricultural economy. While most people think of America as a great industrial nation, many have forgotten that our industrial base is getting old and, in many cases, obsolete. We tend to forget that other nations, such as Germany and Japan, have made great strides in advancing their own industrial economies, and, in many cases, have now passed us.

Our nation still has an absolute economic advantage over the rest of the world in agricultural production. We are still the food basket for the world. We are still able to produce enough food not only to feed ourselves, but also to feed a significant portion of the remainder of the population of the Earth.

For that reason these advancements are important But they must be undertaken with great care. I am concerned that the use of embryo transfers could lead to a situation where only the very best prize cows and bulls would be utilized, and within a few decades we could have lost all of the other genes in the present gene pool. Those who are familiar with dog breeding know what can happen if the strain becomes too inbred. If certain characteristics are overemphasized, you may eventually end up in trouble. More importantly, we may end up with virtually all the animals with a single set of genes, and with no resistance to some new and unknown disease which could wipe out all of the animals.

This entire area raises a number of serious questions, many of which I expect our witnesses to discuss today. It is an area which is bright with promise for all farmers and for all of mankind.

We may be on the threshold of being able to breed and produce "super" animals which can almost be specifically designed to custom fit a special set of needs.

A short time ago we heard from a number of witnesses in a hearing on possible applications of recombinant DNA technology in the plant sciences. It appears that in both cases we are still a long way from being able to clone the plants or animals.

But, as we will hear today, many of the advances made already in the science of animal breeding will have extraordinary impacts on agriculture as we know it today. I want to join the Chairman in welcoming the witnesses, and I also want to thank the Chairman for his interest and for scheduling this hearing. I think that it is of the utmost importance that we in the Congress have the opportunity to keep up to date on this important issue.

Thank you, Mr. Chairman.

Mr. GORE. We have an interesting witness list. We have some of the leading experts in the country with us here today. We are going to have two panels and then a witness from USDA to conclude this session.

Our first panel I would like to call to the witness table at this time, Dr. George Seidel, associate professor of physiology and director of the embryo transplant laboratory at Colorado State University in Fort Collins, Colo.; Dr. Alan Hunter, professor of the department of animal science at the University of Minnesota in Minneapolis; and Dr. Gary B. Anderson, associate professor of animal science at the University of California at Davis in Davis, Calif.

If the three of you would come on up to the witness table.

I would like to ask unanimous consent that immediately following my statement in the record, the statement of our distinguished ranking minority member, Mr. Walker, appear. He is engaged in important business elsewhere. This is the day when we have the reconciliation process, which is one of the most important parts of the budget process. Otherwise, he would be with us.

Without objection, the entire text of the statements of the witnesses will be included in the record. I would like to invite you to proceed, beginning with Dr. Alan Hunter, from the University of Minnesota.

Dr. Hunter, welcome, and please proceed.

STATEMENT OF DR. ALAN HUNTER, PROFESSOR, DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF MINNESOTA; DR. GARY B. ANDERSON, ASSOCIATE PROFESSOR OF ANIMAL SCIENCE, UNIVERSITY OF CALIFORNIA, DAVIS; AND DR. GEORGE SEIDEL, ASSOCIATE PROFESSOR OF PHYSIOLOGY, DIRECTOR, EMBRYO TRANSPLANT LABORATORY, COLORADO STATE UNIVERSITY

Dr. HUNTER. I think probably the best way I can proceed is starting out with the slide set.

While the projector is being set up, I am the leader of a cloning team at the University of Minnesota that is attempting to clone dairy cattle. There are six members of the team. We have a person involved in freezing embryos, Dr. Graham; we have two people involved in collecting eggs and superovulation and embryo transfer; and they are Dr. Fahning and Dr. Crabo; we have a geneticist by the name of Dr. Soffner, who if we have cloned anything can genetically tell us that we really do have an identical offspring; and we have an embryo surgeon, and this is Dr. McKinnell. Dr. McKinnell is famous for cloning frogs. He has successfully cloned frogs. He is the author of the two bibles in the scientific world concerning cloning. My speciality is fertilization, getting sperm together with eggs in order to create a new individual.

[Slide.]

The species we are working with is cattle, and as shown in the next slide, we are looking at cloning from the standpoint of starting with an immature egg and this begins in the ovary.

This happens to be an ovary of a cow. These little spots on here are the places where the eggs reside.

[Slide.]

Now, as shown in the next slide, in the female every egg that a cow will ever have is present in that ovary at birth. The same thing holds true for humans. The same thing holds true for most of our domestic animals.

So we start off during the embryonic period with somewhere around 12 million potential offspring in the form of eggs. By the time birth occurs, we are down to about 500,000. In the case of humans, at menopause, at 45 years of age approximately, we are out of eggs. In the case of a cow, at about 20 years of age we are out of eggs.

This is a section through an ovary showing potential ova, just ready to be released. By just ready to be released, we have devised a technique where we can cut the ovary, just into its surface, and release those eggs.

[Slide.]

As shown in the next slide, those eggs are immature. They contain four times the amount of DNA that we need. When we finally produce amature egg or sperm, we need a 1-X unit of DNA. As far as chromosomes go, it has a 2-N chromosome number. The mature gametes, egg and sperm, are 1-N as far as chromosomes go.

So we have to mature these eggs in the test tube. This is an immature egg. I can tell it right away by this vesicle in the center here. In the test tube, we incubate these eggs after we take away the thing that has arrested their development. In other words, the follicular fluid around them is removed, the eggs are put in culture, and the eggs become activated and start the maturation process.

[Slide.]

When an ovum has matured, as shown in the next slide, we have in the case of a cow, the genetic material inside the egg, surrounded by a membrane (zona pellucida) that keeps the cytoplasm inside.

This is the way an egg would look at the time of fertilization. This germinal vesicle has been broken down. We have the right amount of DNA. We have the right number of chromosomes. Now we are trying to fertilize in the test tube.

In our laboratory in Minnesota, we have successfully accomplished in vitro fertilization of bovine eggs. There are several other laboratories in the United States—Ben Brackett's laboratory in Pennsylvania and First's laboratory in Wisconsin—that have fertilized bovine eggs in the test tube resulting in an early embryos, as shown in the next slide.

[Slide.]

Now here is an early embryo. This happens to be an eight-cell embryo. This embryo might be transferred to an animal. This would be a surgical transfer. The cost would be approximately $2,000, very expensive and not really practical unless you have an outstanding animal.

[Slide.]

However, if we go a little bit further in development, as shown in the next slide, we would have more cells down here. At this particular point, on day 7, this early embryo was in the uterus. We can flush it out of the uterus and transfer it for less than $500. That is the basis of the embryo transfer methodology. They flush the eggs out of the uterus. They are in the uterus from days 5 on, and you can recover them from days 5 to 14 after breeding, and you can transfer them. There is a full commercial business that does this.

What we are doing as far as cloning goes, is to take these early embryonic stages and break the egg into its component parts. Here we have eight cells. There are eight potential offspring from that one individual. Here we have 16 cells. That is 16 potential offspring. Down here we have something like 128 cells or 256 cells. That is the potential for producing identical offspring, using this type of embryo manipulation.

Dr. Willadsen's lab has used it in England to create live calves. Dr. Seidel's lab has used it in Colorado to produce live calves.

When this early embryo starts to go into a form that gives rise to organs, it is much harder to manipulate it. Here we have what we call a blastocyst. These cells outlined in red are is destined to become embryo and the blue cells are destined to become the afterbirth, the placenta. These are the membranes around the calf or the human.

When it gets to this stage, it is much more difficult to manipulate, but using other techniques the genetic material that is present in each of these cells still can be recaptured and, if transferred back into an activated egg, can undergo the full cycle again and result in offspring.

[Slide.]

This happens to be a bovine embryo. Here is the embryo up in here. It is in the placenta, or the afterbirth. This embryo eventually develops into a cow.

[Slide.]

This happens to be Beecher Arlinda Ellen, which happens to be the cow that holds the world's record for milk production, 55,000 pounds of milk. The average cow in Minnesota gives somewhere between 10,000 and 12,000 pounds of milk.

If we could clone this type of dairy animal, we could make a tremendous advance. If we could clone a beef animal that was gaining at extremely fast rates with limited intake, and we could raise the level of protein production. I think this would supply more protein for the world's needs than what we are currently doing.

[Slide.]

The only thing I want to point out about cloning is that we are amplifying something that we consider desirable. Here we are starting with a single cell that contains the genetic material of something that we consider desirable—milk production, rate of gain, size, lack of horns—and we amplify that.

[Slide.]

The technique of cloning involves producing a group of genetically identical cells or organisms, asexually descended from a common ancestor; in other words, we are not putting sexual reproduction into the pattern here. What we are doing is taking an early embryo, taking the cells out of it, amplifying those cells and producing a population of identical offspring containing a desirable trait.

[Slide.]

The nuclear transplantation technique that has been used to successfully clone fish and frogs. It has been used successfully by Karl Illmensee in Switzerland for mice, and it involves taking cells from an early embryo and removing the nuclei from those cells that are destined to become the embryo. Each cell has a nucleus which contains the genetic material. These nuclei are then inserted into an activated egg.

An activated egg is an egg that has become fertilized. It contains the contribution of the male and the contribution of the female. Now we have an egg with the genetic material that we are putting in; the genetic material that was from the father and the genetic material that was from the mother.

We then remove the original genetic material, leaving in place only the genetic material from the transplanted nucleus. The egg undergoes division into 2, 4, 8, 16 cells, right on through to the early stages of differentiation. This blastocyst is then transferred into a recipient animal (using the current embryo transfer methodology that is in place) and it goes to term.

I will make one strong point here. No one has ever cloned an adult cell. That goes for humans, cattle, frogs, and fish. Adult cells have not been cloned. Only embryonic cells have been cloned.

[Slide.]

I would like to end my presentation by making four statements as to where I think some of this research is going to lead.

First, this research is creating embryos in the test tube. When we talk about gene splicing, we talk about taking a gene and putting it into an existing embryo or putting it into an existing cell. The percent takes are very low, but if you are going to do this work in cattle, you have to have a model to generate embryos. Here, maturing the eggs in the test tube allows you to have plenty of potential embryos. If you have a gene that you want to insert into the cattle population, you can use these embryos and fail many times but still end up with enough viable offspring that might make it practical. The place of cloning in this procedure, is that if you have something that is desirable, it allows you to amplify by increasing the numbers of those desirable animals.

As far as the impact that this type of research will have on the production of food and fiber, it would allow you to select for a specific milk composition. We are worried about milk surpluses. We could select for protein and select against fat and select against lactose and eventually get an animal that was high in its protein-producing ability and with very negligible amounts of lactose. Many of the world's population are allergic to lactose. We could then amplify the numbers of those particular animals.

Another big advantage of our embryo work, is that it can decrease generation interval. A heifer bred at 15 months of age has a 9-month gestation period and it comes into milk at 2 years of age. Its milk record is available at 3 years of age. At that time we will say it is a good animal and we want to get more offspring out of it. It will take 6 years from the point I started to finally have more of those animals. That is a long period of time.

If I could take an animal at birth, remove the eggs from it at birth, fertilize them in the test tube and create the embryos, it would allow us to save 3 years. So in other words, we could get the same number of superior animals in a period of 3 years where previously it would take 6 years.

The last thing I want to say is that if recombinant DNA in animals is to be practical, you have to have a workable cloning technique. Without a workable cloning technique, a technique to amplify what you put in, I don't believe the DNA methodology that is currently evolving will ever be practical for dairy cattle or beef cattle production or sheep production.

Thank you.

[The prepared statement of Dr. Hunter follows:]

CATTLE CLONING

A Report to U.S. House of Representatives, Committee on Science and Technology, Subcommittee on Genetic Applications in Animal and Livestock Breeding, July 28, 1982.

by

Dr. Alan G. Hunter, Ph.D.
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University of Minnesota
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Work on cattle cloning--producing from a single fertilized egg many identical cattle--is progressing using two major approaches. The first approach involves dividing an early embryo (2 days to 14 days old) into several pieces. For example, an eight-cell embryo could be split into four parts which eventually result in four identical embryos. Calves have been recently born in England and in Colorado using this approach.

The second approach for cloning cattle involves removing nuclei from cells of the early embryo and transferring these nuclei to fertilized bovine eggs. The parent genetic material of the fertilized egg is then removed, leaving only the genetic material of the transplanted nuclei. The possibility of generating larger numbers of genetically identical cattle resides with this technique. In January, 1981, a Swiss researcher using this technique reported the first authenticated cloning of a mammal (mice). Earlier, fish and frogs had been cloned using nuclear transplantation techniques. To date, NO ADULT ANIMAL HAS EVER BEEN CLONED. All cloning that has produced live animals has originated with embryonic nuclei or tissue.

The Minnesota team that is doing the cloning research is headed by Dr. Alan Hunter, whose speciality is fertilization. He is responsible for creating embryos in the test-tube after maturing bovine ova in the laboratory. Dr. Robert McKinnell, who has successfully cloned frogs, is responsible for the microsurgery on the early embryos. Dr. Melvin Fahning and Dr. Bo Crabo are charged with collecting and transferring cattle embryos. Dr. Fahning besides being a university veterinarian, runs a commercial bovine embryo transfer business.

Dr. Edmund Graham, also on the team, is a specialist on freezing and storing embryos and semen. Since the cloning techniques start with one embryo as a source of genetic material, how good or bad that genetic material is, would not be known until the animals mature. In the case of cattle, this would be approximately 3-4 years later (9 month gestation, milking at 2 years of age, finishing first lactation at 3 years of age). By freezing most of the cloned embryos and testing a few, if superior genetic material had been obtained, the frozen embryos can be thawed and used or their numbers amplified by recloning. The progeny evaluation scheme is currently being used with frozen semen to identify superior sires. The methodology for frozen embryos is currently being used by several embryo transfer businesses. Personally, I feel within 10 years, all major artificial insemination companies will be offering frozen embryos to their customers.

IMPORTANCE

The production of meat and milk are not controlled by a single gene but may be controlled by several thousand genes. Therefore to expect the field of genetic engineering or DNA manipulation to lead to rapid improvement of our livestock is not reasonable. However, the genetic engineers can insert single genes into the existing gene pool of the animal which might help an animal then but when it reproduces this gene is lost or diluted. Cloning is a technique that the animal genetic engineers could use to preserve and amplify the number of their "developed" animals without using sexual reproduction.

The world's record for milk production is 55,661 pounds of milk in one year (Beecher Arlinda Ellen, 1975). The average cow produces about 10,000 pounds of milk. Think what would happen if we could clone that champion cow. It doesn't mean the current milk surplus would be even greater. It means that the farmer will be able to produce more milk using much less feed and labor. It means fewer cows will be needed to produce the same amount of milk. It means higher profits to the farmer.

Protein is the major limiting nutrient resulting in malnutrition. From a global perspective, if developing nations could grow or obtain sufficient protein, much human suffering could be alleviated. Today we can ship embryos of high-producing cattle all over the world to be implanted into their native cows. A cow producing 10,000 pounds of milk produces about 400 pounds of protein. A cow producing 50,000 pounds of milk per year, makes 2,000 pounds of protein without using five times more feed, hence the need to clone superior animals.

Cloning will make the cost of animal research much cheaper, enabling more research to be done per dollar invested. This is because fewer animals would be needed to obtain statistically valid answers. It is about 1,000 times more expensive to do research with cows than with mice.

With identical cloned cattle, the effects of genetics could be readily distinguished from the effects of environment. The Animal Welfare people want to do away with all animal research. With cloning, the number of animals needed for research would greatly be reduced.

CONCLUSION

Using existing research tools such as *in vitro* maturation of oocytes (eggs), *in vitro* fertilization, embryo culture, embryo microsurgery, nuclear transplantation (cloning), freezing embryos and embryo transfer, the commercial business of producing meat, milk and fiber will drastically change from the natural method of reproduction to a man-controlled system. This should result in greater profits for the farmer, more efficient use of labor on the farm and a higher quality final product for the consumer.

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Biography--ALAN G. HUNTER

Alan G. Hunter is a professor of animal science at the University of Minnesota. He joined the staff in 1963.

A native of Pawtucket, R. I., he received his B.S. degree in 1955 and his M.S. degree in 1958 from the University of Rhode Island. He earned his Ph.D. degree in 1963 at Michigan State University.

Hunter was a research assistant at the University of Rhode Island from 1955-1958 and an instructor at Michigan State University from 1958-1963. His specialization is reproductive physiology and biochemistry and he is the author of 80 articles on reproduction.

He has taught courses on the literature of animal science, endocrinology, lactation, reproduction, fertilization, gestation and immunoreproduction. He currently heads a research team that is attempting to clone dairy cattle at the University of Minnesota.

Mr. GORE. Thank you. You got us off to start with a bang, so to speak. That is terrific.

Our second witness on this panel—we will hold the questions, I have a lot of questions as a result of that testimony—is Dr. Gary B. Anderson from the University of California at Davis.

Dr. Anderson, welcome. Please proceed.

Dr. ANDERSON. I would like to make some general comments regarding the nature of gene manipulation in large animals.

Genetic engineering is not a new application to large food-producing animals. I think a very good example of where it has been used successfully is today's high-producing dairy cow. It has been estimated that milk production in dairy cattle is increasing at a rate of about 100 pounds per cow per year, simply due to genetic improvement alone.

This has been brought about by the careful use of artificial insemination. This has not involved simply breeding animals because the breeder likes the looks of the particular male or because the male is from a particular bloodline, as is often done in other species; but rather is the result of some fairly sophisticated analysis of the milk production of the bull's daughters and actual determination of the genetic merit of that bull before he is released for use by dairymen.

Through artificial insemination that bull can produce thousands of offspring, many more than he could possibly produce through natural mating, and thereby improve the genetic pool of dairy cattle in general.

As I said, this is not a new application but has been going on for many years. People have not referred to it as genetic engineering, but it certainly qualifies as such.

Currently there is a lot of publicity being generated about embryo transfer, which presumably is to do for the female what artificial insemination does for the male; that is, increase the number of offspring that a female is able to produce.

Most animal geneticists feel that its impact will probably not be as great as artificial insemination, simply because a female is not likely to produce as many offspring as a male. But it does have the effect of being able to enhance genetic selection on the female side.

There has been tremendous development of the embryo transfer industry from no industry at all 10 years ago to a thriving industry today. I think the reason why the industry has developed so rapidly, probably more rapidly than anyone could have predicted, is that most of the advancements that have been made have been purely technical in nature, the development of techniques which have subsequently been applied.

This includes the development of nonsurgical collection techniques, which means that the animal can be used as a donor, eggs can be flushed from her repeatedly without permanent damage to the animal; nonsurgical transfer, which means that the transfer can be taken right to the farm, a quick and easy technique for putting the embryo back into the recipient; and embryo freezing, which means the embryo can be held indefinitely and transported from here to anywhere in the world. There is an active international market in embryos. In our laboratory we have found that we can successfully sex mouse embryos prior to transfer so that we know which are males and females before they are transferred. We have not yet applied the technique to cattle.

But all of these are simply techniques. There has been very little development of new concepts required in order to use the new technology.

I think when we move from embryos transfer to gene transfer we have a new set of complications. The first successful gene transfer in mammals has been carried out. There has been a report very recently of a gene from the rabbit, which synthesizes a certain blood protein, being transferred to a mouse embryo; the mouse embryos were then transferred to a recipient, young were born, and when the mice matured they were capable of synthesizing the rabbit protein that was coded in the transferred gene.

There are a lot of researchers working on the technical aspects of gene transfer. Researchers are looking at the possibilities of using gene transfer to correct genetic deficiencies. This is very attractive for application to humans.

Certainly it can also be useful in animals, but animal breeders already have a reasonable way of dealing with animals that have serious genetic defects. They just do not let them reproduce.

I think the real application that gene transfer, the very exciting application that gene transfer can have to food-producing animals and one which is going to benefit mankind the greatest, is the transfer of genes that affect the efficiency of food production by that animal; transfer genes that are going to increase the efficiency of milk production in dairy animals or growth rate in meat-producing animals.

But there is another complication because these traits, the production traits, are not under the control of one or only a few genes, but are under the control of many genes. It has been estimated that between 20 to 200 genes may be involved.

The contribution of any individual gene is very difficult to measure and difficult to identify. We can talk about the desirability of transferring these genes, but we do not even know where to begin in identifying the genes that have to be transferred.

Additional work on developing the technique for gene transfer per se is needed, but equally important, support for strong basic research is also needed.

One area which is important is identifying how these production traits are inherited, identifying the genes involved; second, the physiological effect that those genes have on the animal must be characterized.

Until these two types of research are done, we are not going to be able to achieve maximum benefit from gene transfer.

I also have some slides I would like to show.

[Slide.]

They may not fit exactly with the theme, but I show them for two reasons. One is to provide an example of the type of genetic research that is ongoing, attempting to characterize how production traits are inherited in large animals; and second, to provide an example of a manipulation that can be achieved with mammalian embryos in vitro and which affects the ultimate expression of genes in that animal.

These are mouse embryos. They are very similar to embryos of other mammals. As you can see, each embryo is surrounded by the zona pellucida mentioned by Dr. Hunter. These are eight-cell eggs collected about 2 days after fertilization.

Next slide.

[Slide.]

The embryos may be placed in an enzyme to remove the zona pellucida. We do not know exactly what the zona pellucida does, but we do know that we can remove it and still maintain viable embryos.

One thing that the zona definitely does do is keep eggs from fusing.

Next slide.

[Slide.]

If we remove the zona pellucida, we can move two eggs in proximity of one another, and we can actually cause those embryos to fuse.

Next slide.

[Slide.]

This was taken 7 hours after the previous slide, and you can see that even though there are two distinct embryos, the fusion has begun.

Next slide.

[Slide.]

This was taken 20 hours later, and while there are 2 embryos they have formed more of a single embryo.

Next slide.

[Slide.]

This was 20 hours later, the formation of the blastocyst has begun.

Next slide.

[Slide.]

Finally, 48 hours later the formation of a large but perfectly normal appearing embryo is achieved. What is very important is the inner cell mass, the area from which the embryo will develop.

In this embryo there is only one inner cell mass, which means that even though it developed from two embryos, if transferred, the embryo will develop to term, forming one individual, but one that has cell lines derived from both initial embryos.

Next slide.

[Slide.]

We have used this tool to look at the inheritance of genetic traits. A lot of the genetic work that is being done in animals uses laboratory animals as models, and is often carried out coincidently with selection experiments in large animals.

It takes a long time to collect data on the inheritance traits in large animals. This particular mouse is from a line of mice that was selected for 60 generations for rapid growth rate.

This selection experiment took about 10 years to complete. In order to carry it out in cattle, it would take about 120 years. Obviously no one individual could hope to make much of a contribution to the inheritance of growth rate using large animals.

In defense of the use of mice in selection experiments, mice, it appears that the response that one sees in a laboratory animal can be applied on a per-generation basis to what is seen in other animals.

This is a normal sized mouse. The growth line is about twice the normal size. If these individuals are crossed, a dilution occurs in the genes of each line, and much of the growth response is lost.

With cross breeding, half the genetic contribution of each animal is present in each cell. We were interested in seeing what would happen with the complete genotype of both animals within the same individual.

Next slide.

[Slide.]

We use coat color markers. These are some of the many mice of this type that we have produced. It can be seen from the coat color patches that there were contributions from both lines.

[Slide.]

What we are really interested in is on the next slide, and that is the response that we saw in growth rate. We found that if at least half of the cells were derived from the growth line, those animals had a growth rate that was indistinguishable from the pure growth line, indicating that the normal cells of the body were capable of responding to some stimulus from the other cells, which caused them to express a rapid growth rate.

What this has shown is that all of the genes for growth are not needed in all of the cells of the body, but only in a part of the cells. We are now using biochemical markers to determine the contribution of various organs of the body to growth rate and reproduction and to identify the organs or the tissues of the body which are important for expressing the genotypes for the production traits that are of economic importance in large animals.

Thank you.

[The prepared statement of Dr. Anderson follows:]

Genetic Applications in Animal and Livestock Breeding
Prepared for Subcommittee on Investigations and Oversight,
Committee on Science and Technology,
U.S. House of Representatives

July 28, 1982

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Development of today's high-producing dairy cattle has amply demonstrated that genetic manipulation can have dramatic effects upon the productivity of food-producing domestic animals. Estimates of current increases in annual milk production due to genetic improvement alone are as high as 100 pounds of milk per cow. These genetic gains have come primarily through development of programs for identifying genetically superior sires for use with artificial insemination. Additional improvements have recently been made possible through use of embryo transfer techniques to increase the number of offspring from genetically superior females. Although these techniques have been used in research for a number of years, development and commercial application of embryo transfer in domestic animals have taken place only during the past decade. Embryo transfer can be successfully carried out in each of the farm animal species, but only in cattle is there widespread commercial use. Since the primary application has been to enhance reproduction of high priced cows, commercial embryo transfer has paralleled the cattle market. There is growing interest in use of embryo transfer in horses, sheep, goats and pigs.

The development and application of embryo transfer as an important tool in farm animal reproduction have occurred more rapidly than most animal scientists would have predicted. Breakthroughs in nonsurgical collection of embryos made the techniques more acceptable for use in high producing dairy cattle, and development of nonsurgical transfer techniques allowed greater use of on-the-farm embryo transfer. Freezing of embryos has developed from the first report of successful freezing of mouse embryos in 1971 to long-term storage and transport systems and international markets for bovine embryos. Areas in which major developments may be expected in the next five years and which were barely under study five years ago include sex determination of embryos prior to transfer to recipients, *in vitro* fertilization, and cloning (production of identical offspring).

The application of gene transfer to production of large animals is today at a state similar to sexing, *in vitro* fertilization, and cloning five years ago. A few successful beginnings have encouraged researchers to direct their efforts toward improving the necessary technology. Our ability to maintain viable mammalian embryos in the laboratory, outside the maternal system, facilitates efforts to accomplish gene transfer in mammals. The first successful nuclear transplant in mammals was reported in 1981. The nuclei of cells from mouse embryos were transplanted to mouse ova, which subsequently developed into normal individuals that expressed the genome of the transplanted nuclei. The first successful gene transfer in mammals was also reported in 1981. Genes that control synthesis of a rabbit blood protein were transferred to mouse ova that subsequently developed into mice capable of synthesizing the rabbit protein. Such advances point to a time when inherited defects may be corrected by replacing a defective gene with a gene capable of producing a normal phenotype.

Gene transfer may some day be useful in farm animals to improve their genetic quality and food-producing capabilities and thereby increase the efficiency of food production. However, applying gene transfer to food-producing animals is not without complications. Many of the traits of economic importance, such as growth rate in meat animals, milk production in dairy animals, and reproductive performance in all farm animals, are not under the control of a single or even a few genes. Although a few individual genes having a major effect on production functions in farm animals have been identified, production traits appear to be the cumulative effect of many as yet unidentified genes, the contribution of each being difficult to determine and measure.

Animal geneticists and animal breeders have used genetic selection schemes to increase the frequency of genes that positively affect animal production. Shifts in these gene frequencies have occurred, resulting in increased production, without accurate knowledge of the genes being affected. Application of gene transfer in farm animals will certainly require development of the technology to accomplish the physical transfer. However, continued research to identify and characterize the genes affecting production traits is equally important. In addition, the physiological effects of specific genes must be studied so their effect(s) on the animal can be understood. The latter two areas of research are as important as development of techniques for the actual transfer of a gene to an ovum or embryo. Only with emphasis on research in each of these three areas will the application and benefits of gene transfer technology to farm animals be realized.

Mr. GORE. Thank you very much. The next witness on this panel is Dr. George Seidel, associate professor of physiology and codirector of the embryo transfer laboratory at Colorado State University.

Dr. Seidel, welcome. We are glad to have you.

Dr. SEIDEL. Thank you.

I, too, have a few slides and then a short movie and then a few more slides.

I would like to illustrate some of the technology that Dr. Anderson talked about, dealing with microsurgery to embryos. Can we have that first slide?

[Slide.]

These are two calves that were recently born in our laboratory, and they are identical twins, but they are very special kind of identical twins in that they are manufactured identical twins.

We literally took an embryo from a cow and cut it in half and transferred those two half embryos to one cow—although one could transfer one-half to one cow and one to another cow—and got these identical twins.

So that is a form of cloning, although as Dr. Hunter pointed out, cloning of adults is not yet possible. This is cloning of embryonic cells.

Next slide, please.

[Slide.]

This is a micromanipulator, a machine we use to do microsurgery under an embryo, and we will see that in a movie in a little bit.

Next slide.

[Slide.]

This is a cow embryo. Under the microscope at about the 60-cell stage, about a week after the cow was bred. We are holding the embryo with this pipette by the acellular coat around it, the zona pellucida that was mentioned earlier.

Here we have what we call a microsurgical blade. We are about to cut the embryo in half. One can cut them in thirds as well, if one wants to.

Next slide.

[Slide.]

This is a mouse embryo being held by a similar pipette. It is at the one-cell stage. It has just been fertilized. This membrane surrounds the genetic material from the female, the half of the genes that came from the mother of this mouse-to-be, and this membrane surrounds the genetic material from the father, that evolved from the sperm.

The gene transferring experiments involve injecting genes directly into these nuclei. Here you can see a pipette in one of those nuclei.

I would like to show a movie of these sorts of techniques. Could we have the movie, please?

[Movie shown.]

Part of this movie was made at Yale University while I was there on sabbatical leave and part of it is from our laboratory at Colorado State University. It involves mouse embryos and cow embryos.

This is the micromanipulator I mentioned. The principle is that one can manipulate equipment under the microscope. A large movement with the hand is translated to a small movement under the microscope, and you see these microsurgical devices here, which we will see through the microscope in just a minute.

The embryos are in little droplets of medium that are under a layer of oil to prevent the medium from evaporating.

This is through the microscope now. Again, these are one-cell fertilized mouse embryos. With this holding pipette we can grab a hold of an embryo, we can give it an injection, remove the injecting pipette, grab a hold of another embryo—I call this my herding embryo sequence—let go of that embryo, and so on.

This is a higher power version of the embryos under the microscope. Again, a little bit of fooling around here to illustrate that one can in fact manipulate the embryos. One does that by just applying suction to these pipettes and moving them in the right plane, applying suction, getting the embryo just exactly where you want it.

This is a micropipette that could be used to inject genes into a nucleus. In fact, that experiment is done exactly this way. In this series, though, we were removing the nucleus. This is the male genetic material, and it has been removed from the ovum.

I call that unfertilizing an egg. The sperm worked hard to get it in and then we took it out a couple hours later. It did not kill the embryo to do the injection or to remove the male genetic component after embryonic development started.

I am going to blow a droplet of oil out there. That is just to illustrate that the system is filled with oil.

This is a second sequence of the same thing. Again, I am removing—there the holding pipette let go—the male genetic component. One could just as well remove the female component or one could do various other things as well, so the genes that the sperm put in would be in that particular nuclear membrane.

Here you can see very nicely the two genetic contributions to the mouse that would develop from these pronuclei. The little things inside are known as nucleoli.

A very special kind of optical system is used here to amplify the biological components. It is called Nomarski optics.

Now then we have a final sequence of microsurgery of the type we have been illustrating. There are going to be some problems with this one. When we made this movie we thought we shouldn't show all the easy things but show some of the problems we can get when we are removing pronuclei.

There is a piece of dirt sticking to that pipette, and a little piece of the wrong sort of thing in one's equipment can cause all sorts of problems. This was just focusing up and down through the embryo to illustrate that one has to be in the right focal plane.

So I am going in to remove that pronucleus, that nuclear material from the male, and because of the obstruction in the pipette, it is not possible to get a good grip on the nuclear membrane. So I go back and try harder.

I am persistent enough such that the suction from the holding pipette is lost, and I go back again, and that just proves that there is more than one way to skin a cat.

I again point out that it does not kill the embryo. It will go on dividing with only half the genetic material, but it will die eventually. This is the same sort of thing with the larger pipette where we suck the whole pronucleus into the pipette.

This is the sequence on how we make identical twins in the cow. We start with an unfertilized abnormal embryo and first crack this zona pellucida. This is an abnormal ovum that one could get from any nondescript cow or even a normal cow that just produced a bad embryo.

We then remove the bad cell from the shell, the zona pellucida, so this illustrates the acellular coat around the embryo very nicely. Then we take a normal embryo and cut it in half.

What we are doing in this process is taking a good, normal embryo and an abnormal ovum and we are going to make two good ones out of a good one and a bad one. We are going to use the zona pellucida, the acellular coat in the one, to be a protective shell for half the embryo that is taken from the other.

The principle I think you can see is to cut the embryo in half and then suck out half of the cells and put it into the zona pellucida that we evacuated, and in that way make two half embryos out of a good embryo and a bad embryo.

Let's see the slides.

[Slides.]

Now you might question the wisdom of unfertilizing an ovum, why take the genetic material out? This is a rabbit ovum that has been fertiiized by two sperm. This is the female genetic material, the male genetic material from one of the sperm is here—and those two are snuggling each other, which pronuclei do—and the genetic material from the other sperm is here.

This embryo would be considered a triploid and it would eventually die. It has too much genetic material. You have got to have just the right amount. We can correct this situation—and this occurs with the frequency of perhaps a couple percent in cattle—by removing one of these male pronuclei so it can get back to normal.

Now, in our lab we are not interested in that experiment, but we are interested in the experiment or the technique where we remove the female pronucleus. So what we have then is an egg fertilized by two sperm, with the female genetic component removed, and if we then transferred that embryo, we would get an individual that has two fathers and no mother, genetically.

If the two sperm were from the same male, one would be able to cross a male with himself. If the sperm were from different males, one could cross one male with another. If the two sperm were X sperm, one could even get a female from the procedure. So one can cross one male with another male and get a female.

We are interested in that sort of experiment for research purposes, but also in the case of dairy cattle where we can identify bulls very accurately for milk production of their daughters we could cross two bulls.

Next slide.

[Slide.]

Another thing one can do with this splitting embryo methodology is to sex half of the embryo. These are two of the five cows that I happen to own. This is Princess and this is Louise. Louise is carrying a pregnancy from Princess, and I know that she is going to have a heifer calf. We did it by this technology.

Next slide.

[Slide.]

This is another anecdotal point. We freeze a lot of embryos in our laboratory. This is an Angus-cross range cow in Montana to whom we transferred a frozen Simmental embryo last year. These techniques can be used in some circumstances.

I guess hopefully the idea is to produce something like the moo-baa-quack, an animal that has desirable combinations of cattle, sheep, and ducks combined into one.

Thank you.

[The prepared statement of Dr. Seidel follows:]

GENETIC ENGINEERING WITH LIVESTOCK
prepared for Subcommittee on Investigations and Oversight
Committee on Science and Technology
U.S. House of Representatives.
July 28, 1982

George E. Seidel, Jr.
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Genetic engineering and gene transfer are terms that can be used in many contexts. For example, one can think of them on the basis of a cell, a whole animal, or a population. One area of genetic engineering, which has considerable potential for improving livestock production, concerns a wide range of manipulations that can be performed on an ovum between the time when it is released from the ovary (ovulation) and the the time when it makes intimate contact with uterine tissues (implantation). During this interval the ovum is fertilized, and thus becomes an embryo. The ovum and embryo are self-contained and may successfully be removed from the reproductive tract and maintained in culture in test tubes. Some examples of the manipulations that can be performed include: 1) embryo transfer, which can be simply the removal of an embryo from the reproductive tract of one female and transfer to that of another, 2) sex determination by examining individual chromosomes (or screening for morphologically abnormal chromosomes), 3) transfer of pronuclei, nuclei, or cytoplasm, based on fairly straightforward microsurgery, and 4) insertion of DNA sequences into the genome of a fertilized ovum, a process that is still in early developmental stages, but promising. These techniques of genetic engineering vary to a considerable extent in requirements in terms of the amount of and costliness of equipment, the level and number of technical skills, the amount of time to carry out the procedures, and the tolerance of the embryos to manipulation.

Many of these genetic engineering techniques are frequently a step or two behind the demand on the part of livestock breeders, and many findings can be applied immediately, in some cases even before they become cost effective. For example, producing identical twins from embryo splitting was first reported in 1978 by Moustafa and Hahn in Germany with mice. The technique was improved by Willadsen in England in 1979. In 1981, Williams et al. at Colorado State University simplified the methodology for cattle and within a year a pair of identical twin pregnancies from split embryos was sold in a public sale for $47,000.

Recent advances have enabled the following technical capabilities, which are currently offered as services by commercial embryo transfer companies:

1) Surgical and nonsurgical embryo recovery and transfer to obtain more progeny from valuable females, to obtain progeny from infertile females, to introduce new genes into specific pathogen-free herds, to increase the population base of rare or endangered breeds or species, and to test individuals for Mendelian recessive traits.
2) Cryopreservation of embryos (40% pregnancy rate following freezing, thawing and transfer of bovine embryos, which is lower than the 60-65% rate with fresh embryos, but already commercially useful).
3) Splitting of embryos to produce identical twins (50% pregnancy rate per half embryo or an average of one calf per original embryo instead of the usual 60-65% pregnancy rate per whole embryo).
4) Import or export of fresh or frozen embryos (thousands of bovine embryos have already been exported from the United States).
5) Induced twin pregnancy by transfer of an additional embryo (70% pregnancy rate; 50% twinning rate). This has tremendous potential for obtaining more beef per unit of feed. Currently it is not commercially viable, but would become so if feed becomes scarce or beef prices rise substantially.

There are many more techniques coming along that will be put to use by some livestock producers when procedures are improved. These include:

1) Sexing semen and embryos, which will greatly improve livestock efficiency if sexing is inexpensive and efficacious.
2) In vitro fertilization, which can be used to circumvent some kinds of infertility, and be used for testing fertility.
3) Fertilizing one ovum with another ovum instead of with a sperm. This would permit crossing a female with another female or with herself.
4) Fertilizing an ovum with two sperm and removing the female genetic material microsurgically. This would permit crossing one male with another male or even with himself. If the two sperm had X chromosomes, females would even result.
5) Producing large numbers of identical multiplets by nuclear transplanting cells from older embryos. This ia a form of cloning.

6) Injecting new genes into 1-cell embryos. This has already been done with limited success in mice. This is the ideal stage for accomplishing gene transfers as each cell in the body of the animal that results gets its genetic information from this first cell.

There are many more things to be done. For example, although gene transfer technology is improving rapidly, in most cases we do not know enough about the basic biology of domestic livestock to know which genes are important for improving economically important traits, or where in the genome of the animal useful genes might be located. Until useful genes are identified, they are not useful in these contexts. A second example of our ignorance concerns superovulation, treatment of females with hormones so that they ovulate more ova than normal. Sometimes this procedure works very well, and sometimes it is a complete failure. This variability is one of the most critical problems for most of the technology described in this report. Since we do not understand how ova normally mature and ovulate, it is not surprising that we do not understand the system when we impose hormonal treatments. As we come to understand the nature of various life processes in more detail, it will become easier to regulate them, and undreamed of applications will emerge.

It is probably worth mentioning a few of the spinoffs of such technology. A good example is splitting embryos. Currently the major practical application is to produce more calves per embryo recovered, about 50% more. The fact that many of these are identical twin pairs, however, also raises exciting possibilities for improving experiments. If one member of the pair is assigned to treatment A, and the second to treatment B, and this is continued with several sets, the genetic variation in the experiment is completely controlled. Such a scheme reduces animal requirements for experiments, and in some cases costs can be cut to less than half. Another fringe benefit of improving livestock production is that fewer animals are needed, thus reducing feed requirements and resulting in less pollution from animals.

What should the role of the U.S. Congress be in these matters? First, although good progress has been made, much more remains to be done. The biggest void seems to be our lack of understanding of basic biology. Traditionally, research of a basic nature has not been funded to a great extent by agencies such as U.S.D.A., and perhaps a modest effort in this respect would be advisable. Clearly, such research is of benefit to the entire country, however, especially in the context of human health.

One suggestion would be to expand the competitive grants program of U.S.D.A. Currently that program is small, but even more disconcerting is that funding is limited to certain defined areas. If someone comes up with a good idea that is not within these defined areas, there is no mechanism available for U.S.D.A. funding. A modest program of competitive grants to take advantage of promising ideas that do not fit into preconceived slots would seem desirable.

One final option is to initiate directed National programs to take advantage of special opportunities. The French government has such a program in the general area of manipulating embryos of domestic animals, and it is likely to be very effective. An example of an area which might be ripe for this approach is sexing semen.

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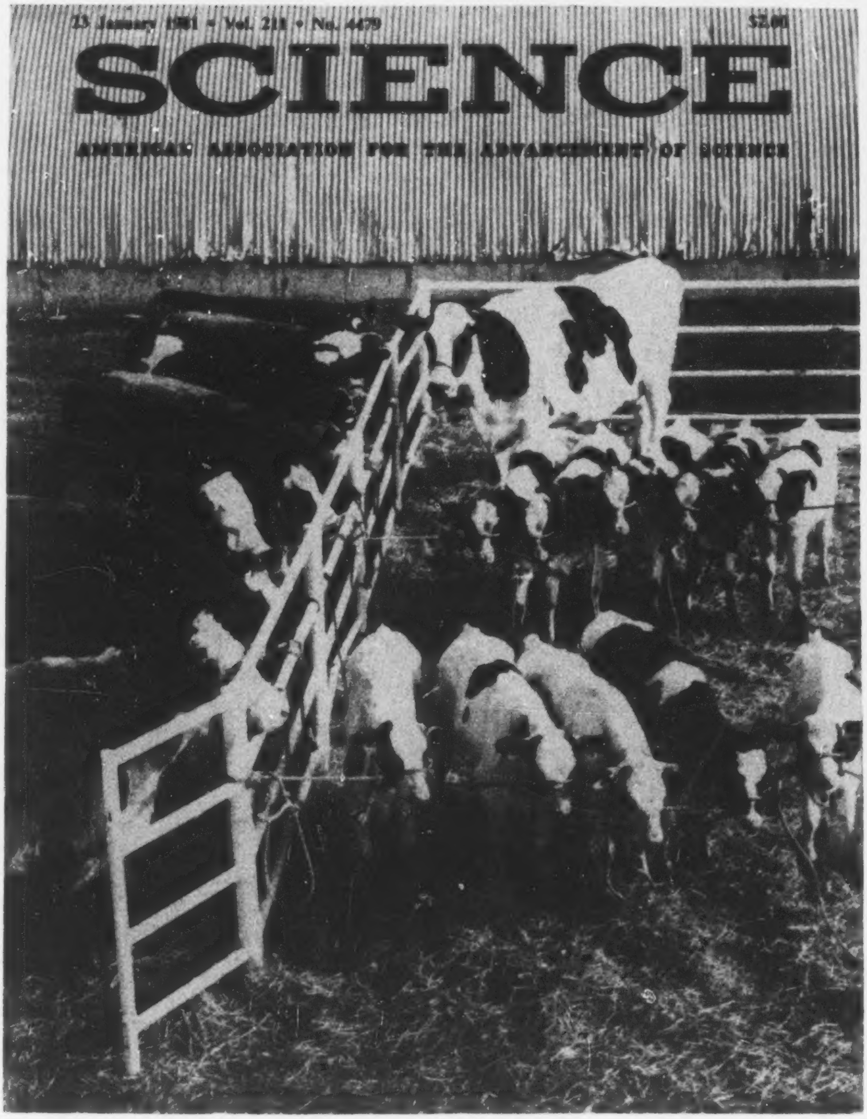

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Superovulation and Embryo Transfer in Cattle

George E. Seidel, Jr.

Summary. About 17,000 bovine pregnancies were produced by superovulation and embryo transfer in North America in 1979. The major use of these techniques is to increase the reproductive rate of valuable cows. Other applications include circumventing infertility, exporting embryos, and testing potential carriers for Mendelian recessive alleles. Cryopreservation of embryos is beginning to be used commercially, and sexing embryos before transfer may soon become routine.

A multimillion dollar industry centered on recovery, in vitro culture, and transfer of bovine embryos has evolved over the last decade. This article describes the techniques being used, examines commercial applications, and describes developing techniques and applications that are likely to be used in the near future.

Gestation in cattle lasts just over 9 months; usually a single offspring is born, but twins result from a few percent of pregnancies. Cattle are not considered to be seasonal breeders but are often managed to reproduce seasonally, calving before the time of the year when the cheapest forage is available. Genetic progress is limited by the low reproductive rate and long interval between generations, an average of 6 to 7 years (*1*). Thus, during a 40-year career, a cattle breeder works with an average of only six or seven generations. Moreover, it takes years to increase cattle numbers in response to economic incentives. This is responsible, in part, for the 10-year cycle, in the United States, of high and low beef cattle numbers and the consequent fluctuations in beef prices (*2*). Similarly, it takes decades for cattle numbers to recover after depletion due to war, famine, or social upheaval (*3*).

Ovum and egg are the general terms used to refer to the female gamete and early embryo. The ovulated ovum is technically termed an oocyte which, upon fertilization, becomes an embryo, and when the major organs form, a fetus (Fig. 1). Embryo transfer refers to placing an embryo into the lumen of the oviduct or uterus (*4*). In a broad sense, however, embryo transfer has come to mean the sequence of steps for moving embryos from one female to another, including superovulation, embryo recovery, and storage of embryos in vitro. The donor is the genetic mother from which embryos are recovered; the host or surrogate mother is called a recipient.

The first successful embryo transfer was performed in rabbits 90 years ago at Cambridge University (*5*). Since that time, embryo transfer has been used for research in more than a dozen species, although extensively only in the mouse, rabbit, sheep, and cow.

The first successful bovine embryo transfer was reported in 1951 (*6*). Over the next two decades a number of researchers repeated this feat, culminating in the high success rates achieved by Rowson and his colleagues (*7*). In the last decade, the development of new drugs and techniques coupled with demands for embryo transfer services by the cattle industry have led to significant advances (*8*). Two human infants, a girl and a boy, were born after in vitro fertilization and return of the embryos to the original donors by transfer (*9*).

Commercial embryo transfer has been centered in North America, but the size of this industry had not been documented until a systematic survey was undertaken of all companies known to offer such services in the United States and Canada (*10*). The survey, summarized in Table 1, is an underrepresentation of the volume of business both because two firms did not respond and because some were probably overlooked. However, it is likely that more than 90 percent of the commercial embryo transfer activity in North America is included. Data for 1979 are estimates based on 80 to 90 percent of the year's business. Although the volume of business appears to be very large, the most noticeable aspect is the 80 percent increase in pregnancies between 1978 and 1979. This may be explained in part by the increase in cattle prices after the liquidation of breeding herds in 1976 and 1977 when cattle prices were low (*2*). However, improved technology and acceptance of embryo transfer techniques have also contributed. Embryo transfer has become a $20-million-a-year industry (*11*); essentially no commercial activity existed before 1972.

Commercial activity outside North America has been less extensive. Over the past decade, several European firms have operated commercially for brief periods (*12*), and several centers have become more firmly established. Governments in Western Europe, particularly England, France, Ireland, and West Germany, have supported considerable research in embryo transfer. This has stimulated commercial application worldwide, especially in Canada and the United States.

Embryo transfer is a larger industry in Australia and New Zealand than in Europe (*13*), although it declined sharply a few years ago when cattle prices decreased, and it is only now recovering. Interest in embryo transfer is high in dozens of other countries, but the amount of commercial activity is probably small.

Current Technology

Treatment of cattle with gonadotropins leads to ovulation of numerous ova instead of the usual one. The basic mechanisms of superovulation are understood only superficially (*14*). Gonadotropin treatment is usually initiated between days 9 and 14 of the estrous cycle (estrus is day 0), causing ovarian follicles to grow (*8*, *15*). Two or three days after the start of treatment prostaglandin $F_{2\alpha}$ or an analog is injected to terminate the luteal phase of the estrous cycle prematurely by lysing the corpus luteum; about 2 days later estrus occurs. Estrus lasts

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about half a day; ovulation occurs about one-half day after the end of estrus; and fertilization probably occurs a few hours after ovulation (*8*).

Before prostaglandins became available, superovulation was initiated about 4 to 5 days before the end of the estrous cycle, a time that could not be estimated accurately. Availability of prostaglandin $F_{2\alpha}$ has improved the efficacy of superovulation and has also provided flexibility in scheduling donors. Few would have predicted that basic research with prostaglandins would eventually result in such an application (*16*).

Because the best bulls are usually propagated only with frozen semen, artificial insemination is used routinely for valuable cows. Sometimes mixtures of semen from two or three bulls are used with superovulation, and the progeny are sorted out after birth on the basis of blood type.

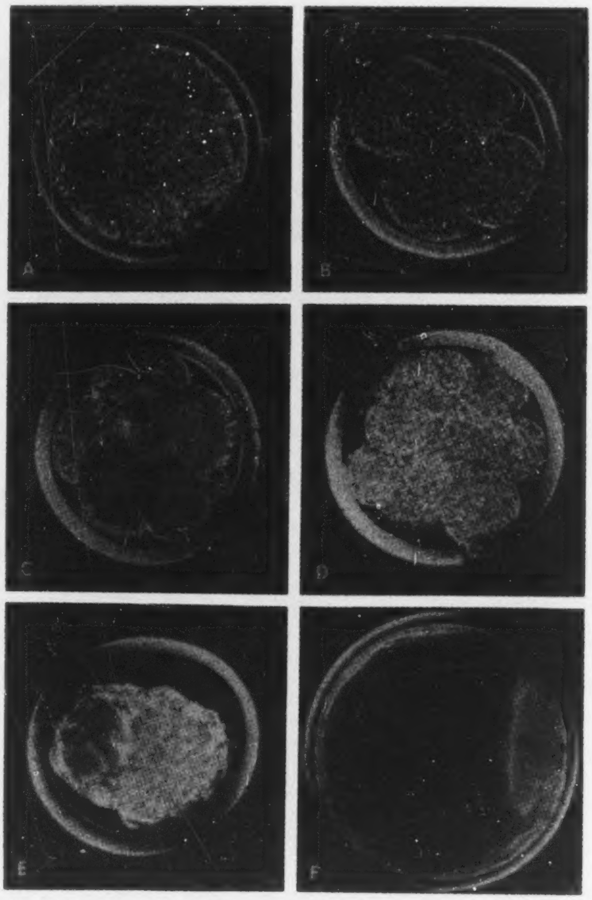


Fig. 1. Bovine ova. (A) Unfertilized oocyte recovered from the ovary. Follicle cells were removed mechanically. The outside layer is the zona pellucida. (B) Four-cell embryo recovered from the oviduct on day 2 of pregnancy (estrus = day 0). Note perivitelline space between the cells and the zona pellucida. (C) Eight-cell embryo recovered from the oviduct on day 3 of pregnancy. (D) An 8- to 16-cell embryo (morula) recovered from the oviduct on day 4 of pregnancy. (E) Very early blastocyst stage; approximately 60 cells recovered from the uterus on day 7 of pregnancy. The inner cell mass from which the fetus will develop will form to the right. (F) Expanded blastocyst with > 100 cells recovered from the uterus on day 8 of pregnancy. The inner cell mass is to the right. All photomicrographs were taken with differential interference contrast (Nomarski) optics. Magnification approximately ×300.

Bovine embryos move from the oviduct to the uterus 4 to 5 days after estrus (3 to 4 days after ovulation), although in superovulated cows a few remain in the oviduct through day 7 (*17*). A high percentage of embryos can usually be recovered nonsurgically from the uterus six or more days after the beginning of estrus (*18*, *19*). Recovery of embryos from the oviduct requires surgery and, therefore, is recommended only in certain cases of infertility (*20*, *21*).

Procedures for obtaining, storing, and transferring embryos are illustrated in Fig. 2. To recover embryos, an 18-gauge Foley catheter is inserted through the cervix into the uterus by palpating through the wall of the rectum with one hand as is done for artificial insemination. The latex catheter consists of three channels for inflow, outflow, and inflation of a balloon-like cuff that prevents the escape of fluid after insertion (Fig. 2). Each uterine horn is filled and emptied five to ten times with 30 to 200 milliliters of fluid each time, according to the size of the uterus. The embryos are flushed out with this fluid into large graduated cylinders. After 30 minutes, embryos settle and can be located under a stereomicroscope by searching through an aliquot from the bottom of the cylinder. They are then stored in small containers until transfer.

Embryos from the one-cell to the early blastocyst stage (7 to 8 days after estrus) are between 120 and 140 micrometers in diameter exclusive of the zona pellucida (Fig. 1) (*8*, *22*). Between days 8 and 10, they double in diameter, hatch from the zona pellucida, and then grow to 20 centimeters or more in length by day 18 (*23*). Since bovine embryos form no intimate attachment to the uterus before day 18, they can be recovered nonsurgically until this time, although they are increasingly prone to damage after day 14. It appears that a larger number of normal embryos can be obtained nonsurgically 6 to 8 days after estrus than at other times.

In vitro culture and storage of bovine embryos. The ability to keep embryos in vitro provides considerable flexibility. For example, it is not unusual to recover embryos from a dairy cow and then fly hundreds of miles to a recipient herd. Bovine embryos, particularly those at the morula and early blastocyst stages, continue development in vitro at 37°C for 24 hours in nearly any properly buffered and balanced salt solution supplemented with blood serum or bovine serum albumin; however, for periods greater than 24 hours, a complex medium, such as Ham's F-10 plus 10 percent fetal calf serum, appears best (*24*). Pregnancy rates for embryos cultured up to 24 hours are similar to those of embryos cultured for only a few hours, but there may be more subsequent embryonic death after transfer of embryos cultured for 24 hours or more (*25, 26*).

Although, for commercial purposes, embryos are usually kept in vitro for less than 10 hours, a pregnancy rate of 44 percent has been reported for embryos collected in Canada and transferred to recipients in Europe 18 to 33 hours later (*27*). Embryos also continue to develop normally when stored in the oviduct of a living rabbit for 2 to 4 days, and pregnancy rates are high when they are recovered and retransferred to the bovine (*28*).

Embryos are often kept at ambient temperature (15° to 25°C) between collection and transfer. Storage of embryos at 0° to 10°C halts embryonic development until they are again warmed to 37°C. Pregnancy rates with bovine embryos stored in this manner for a day or two are reduced somewhat (*29*); occasional pregnancies have resulted after transfer of rabbit and sheep embryos stored for up to 10 days at 10°C (*30*).

For long-term storage, embryos are frozen to −196°C in liquid nitrogen. About one-third of bovine embryos are killed or severely damaged after freezing and thawing, but pregnancy rates following transfer of the other two-thirds are not much lower than those with fresh embryos (*26, 31*). Despite the losses, freezing is sometimes used commercially when recipients are not available. Just as nearly all bovine semen used for artificial insemination in North America is frozen despite the fact that freezing and thawing kills half of the sperm (*32*), most bovine embryos probably will be frozen between collection and transfer if losses due to freezing and thawing can be reduced to less than 20 percent.

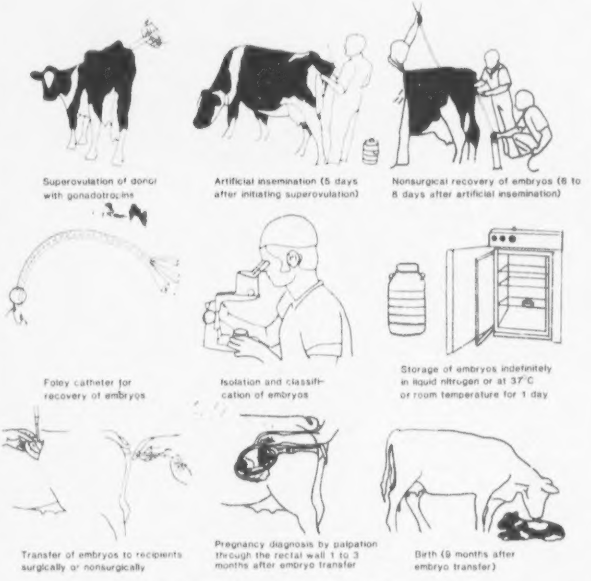
Embryo transfer. Embryo transfer technology is commercially viable because reproductive rates of valuable donor cows can be increased through less valuable recipient cows. A common misconception is that the recipients are undesirable cattle rejected for other purposes. In fact, though less valuable than donors, recipients usually are superior to the general cattle population. The successful recipient must be fertile, free from disease, and able to give birth to a calf without complications. With many systems of management, it is desirable that she also produce sufficient milk to ensure that the calf grows at an optimal rate. Easily the largest single cost of embryo transfer is procuring suitable recipients and maintaining them in a nonproductive (nonpregnant) state until embryos are available for transfer.

Recipients must be at the same stage of the estrous cycle as the donor. Asynchrony between the donor's and the recipient's estrous cycles of 1 day results in pregnancy rates similar to those with exact synchrony; however, pregnancy rates plummet when the estrous cycles of donor and recipient are more than 2 days out of phase (*26, 33*). The requirement for estrous-cycle synchrony necessitates maintenance of herds of hundreds of cows to provide sufficient synchronous recipients for each superovulated donor. Manipulation of the estrous cycles of recipients with drugs such as prostaglandin $F_{2\alpha}$ is useful under some circumstances (*34*). Unfortunately, drugs for estrus synchronization must be given before actual recipient needs are known. The great advantage of frozen embryos is that embryos wait for recipients, instead of the converse.

Embryos are normally transferred to the uterus. Currently, nearly half of the commercial embryo transfers are performed nonsurgically, and this method is rapidly replacing surgical ones. It is accomplished with the same equipment that is routinely used for artificial insemination; however, the embryo is deposited further into the uterus than semen, and much more care is required to prevent introduction of microorganisms, since the progesterone-dominated endocrine environment makes the uterus especially prone to infection. In contrast, it is extremely difficult to infect the uterus when cows are routinely inseminated because of estrogen domination at that stage of the estrous cycle (*35*).

Table 1. Companies producing pregnancies by bovine embryo transfer in North America. Pregnancies were confirmed between days 60 and 90 of gestation. The 1979 data were estimated between October and November. In Canada, pregnancies totaled 2020 in 1978 and 3697 in 1979. In the United States, pregnancies totaled 7149 in 1978 and 13,008 in 1979.

Pregnancies (No.)	Companies (No.)	
	1978	1979
> 1000	1	4
500 to 999	5	7
250 to 499	10	11
100 to 249	6	7
< 100	13	16



Superovulation of donor with gonadotropins

Artificial insemination (5 days after initiating superovulation)

Nonsurgical recovery of embryos (6 to 8 days after artificial insemination)

Foley catheter for recovery of embryos

Isolation and classification of embryos

Storage of embryos indefinitely in liquid nitrogen or at 37°C or room temperature for 1 day

Transfer of embryos to recipients surgically or nonsurgically

Pregnancy diagnosis by palpation through the rectal wall 1 to 3 months after embryo transfer

Birth (9 months after embryo transfer)

Fig. 2. Synopsis of bovine embryo transfer procedures.

Pregnancy rates with nonsurgical transfer are lower and more variable than with surgical transfer (*36*). For surgical transfer, the uterus is exposed either through an incision in the flank for which local anesthesia is used or through an incision between the umbilicus and udder for which general anesthesia is used. A puncture wound is made in the anterior portion of the exposed uterine horn ipsilateral to the ovary containing the developing corpus luteum (site of ovulation), and the embryo, in about 0.1 ml of medium, is transferred into the uterine lumen through a fine pipette, several centimeters long and about 1 millimeter in diameter at the tip. If the embryo is transferred to the uterine horn contralateral to the corpus luteum, pregnancy rates are reduced by half (*36, 37*).

Pregnancy rates with normal embryos from superovulated cows range from 30 to 60 percent with nonsurgical transfer and from 50 to 70 percent with surgical methods (*8, 15, 26, 36*). The latter rate is slightly higher than the pregnancy rates of 50 to 60 percent with routine artificial insemination (*38*), in part because unfertilized ova and abnormal embryos are discarded before transfer.

Tens of thousands of calves have been born as a result of embryo transfer, and there is no evidence of increased abnormalities. However, no systematic comparison has been made between calves produced by embryo transfer and those produced conventionally. Findings (*39*) from survey data of 178 pregnant recipients showed four abortions between 90 days of pregnancy and term, seven deaths of newborns (including one abnormal calf), and seven additional deaths before weaning. Thus 160 (90 percent) of the 178 pregnancies resulted in normal, weaned calves; this weaning rate is similar to that for routine cattle husbandry in well-managed herds (*40, 41*). The sex ratio was 51 percent male, which is normal. There were no identical twins, although there are anecdotal accounts of identical twin calves resulting from transfer of single embryos. Statistical documentation of normal offspring and sex ratios was not a prerequisite for accepting embryo transfer technology by the cattle industry.

Rates of success. When cows are inseminated without superovulation, it is possible for skilled personnel to recover the ovum nonsurgically in 60 to 70 percent of the cases (*19, 42*). About 10 percent of the ova recovered are unfertilized and another 10 percent are morphologically abnormal. Thus, a normal embryo is obtained about 50 percent of the time. Pregnancy rates after transfer of such embryos are close to 70 percent (*15*); therefore, the probability of getting a calf from each attempt is about 35 percent. A cow ovulates about 17 times a year, although estrus would probably only be detected about 15 times; thus, about five calves per year could be produced by embryo transfer without superovulation.

With commercial embryo transfer, donors are usually superovulated. The best treatments result in six to seven normal and two to three abnormal or unfertilized ova per normal cow treated; three to four pregnancies result on the average when embryos are transferred surgically (*15, 26*). To illustrate success rates, repeatability, and variability with current procedures, data from donors at the clinic at the College of Veterinary Medicine and Biomedical Science at Colorado State University are presented in Table 2.

From three courses of superovulation with follicle-stimulating and luteinizing hormone (*15*) of each of 32 apparently normal donors (*43*), 328 pregnancies resulted. Embryos were recovered nonsurgically and transferred surgically. The donors were beef cows from 1 to 14 years of age. They are fairly representative of the beef cattle in embryo transfer programs (*44*). Although superovulation and embryo transfer are frequently used with dairy cattle, similar data are not available since it is rare to keep normal, fertile dairy cattle nonpregnant for three courses of superovulation because pregnancy and parturition are required every 12 to 13 months for optimum milk production.

Table 2. Distribution of pregnancies from donor cows. Pregnancy was confirmed by palpation through the rectal wall between days 90 and 100 of gestation.

Donors		Pregnancies from superovulation (No.)		
Age (years)	Breed	First	Second	Third
1	Simmental	4	7	8
1	Simmental	5	0	9
4	Limousin	0	0	1
2	Hereford	5	1	4
2	Pinzgauer	6	1	0
2	Pinzgauer	4	8	20
13	Angus	4	2	8
10	Hereford	2	0	5
9	Hereford	9	3	7
1	Simmental	7	0	0
1	Simmental	2	1	0
4	Charolais	7	9	0
7	Brangus	2	0	1
2	Simmental	5	11	4
6	Hereford	1	11	5
8	Hereford	2	2	0
5	Hereford	2	0	1
14	Hereford	12	0	1
12	Hereford	0	3	2
2	Hereford	3	1	4
4	Angus	3	0	2
7	Hereford	4	7	3
5	Angus	4	2	0
5	Angus	4	3	0
5	Angus	4	9	0
4	Simmental	1	1	0
5	Angus	0	7	2
4	Hereford	0	4	8
10	Angus	0	6	0
1	Simmental	0	2	0
5	Angus	3	6	0
3	Simmental	3	4	14
			Average	
		3.4	3.5	3.4

Perhaps the most obvious characteristic of the data in Table 2 is variability. The data are not normally distributed and, therefore, many standard statistical descriptions are not appropriate. For example, calculated standard deviations for second and third superovulations are larger than the means, resulting in coefficients of variation greater than unity. A second characteristic of the data is the lack of predictability from one superovulation to the next; the repeatability, calculated from variance components was only 1 percent. The low repeatability of the number of pregnancies is partly due to accumulation of variability in superovulation, fertilization rate, efficiency of embryo recovery, availability of suitable recipients, and other factors.

Mean numbers of pregnancies were similar for all three courses of superovulation. However, half of the donors had fewer than two pregnant recipients from third superovulations, in contrast to one-fourth of the donors for first superovulations. Data from the third superovulation must be interpreted with caution because of the two high values of 14 and 20 pregnancies.

Although the number of animals is limited, the data are typical (*8, 26*). Under commercial conditions, one can reasonably expect an average of three to four pregnancies from one superovulation and about ten pregnancies from three, but results for an individual cow are unpredictable. Typically, one-half of the pregnancies are from one-fourth of the donors. This makes it extremely difficult for the farmer to plan breeding programs and for the embryo transfer unit to schedule workloads. The major determinant of the number of pregnancies is the number of normal embryos obtained, although pregnancy rates among donors also vary widely. Thus far, the variability has not been explained, although age, breed, nutrition, and season have been marginally implicated (*8, 45*).

Superovulation is not recommended until a donor exhibits a normal estrous cycle, characterized by two estrous periods 17 to 24 days apart. Because estrous cycles are often modified temporarily by superovulation, an interval of 2 months or more is usual between superovulations. For the donors in Table 2, the median interval between the first and second superovulation was 78.5 days and between second and third superovulations, 82 days. The median elapsed time between arrival of donors at our embryo transfer unit and the first superovulatory treatment was 46.5 days. Thus, nearly 7 months were required for three superovulations. On the average, however, reproductive capacity was amplified more than tenfold. The yield can be increased further by the transfer of unsuperovulated embryos recovered before and between superovulations while normal cycles are being established. Recovery of a single ovum is not usually attempted because of greater expense per pregnancy relative to superovulation; however, it was attempted from about 60 percent of the eligible estrous periods of the donors in Table 2, and 15 extra pregnancies resulted.

A number of cows have been superovulated four or more consecutive times without an intervening pregnancy (*46*). Some of these donors continued to respond, but data are insufficient to recommend superovulation more than three times in a row.

Cost of embryo transfer. Embryo transfer units currently charge about $2000 per pregnant recipient. When the various other costs are taken into account, and the value of the recipient is deducted, it turns out that calves must be worth about $2500 each at 6 months of age for embryo transfer to be profitable (*47*). About $1500 of the cost is attributable to embryo transfer; the remainder represents conventional costs.

Although the majority of bovine embryo transfer work is still done at embryo transfer centers, there is a definite trend toward doing both recovery and transfer on the farm, where, under certain conditions, embryo transfer costs may be much lower. As deep-freezing methodology for embryos improves, most embryos will be transferred on the farm and costs will plummet.

Commercial Applications

By far the major use of embryo transfer is to increase the reproductive rate of valuable cows. Although it is impressive for a cow to have ten calves per year, it is extraordinary that some cows have had more than 50 calves in a 12-month period. This must, of course, be weighed against those that have only a few, despite persistent effort.

Donors are usually chosen on the basis of the anticipated commercial value of the calves rather than on the basis of direct genetic considerations. At a recent auction, a bull calf produced by embryo transfer was the top selling animal at $131,000; the third highest priced animal at $27,000 was a maternal sister to the bull, also from embryo transfer. However, this technology can lead to such rapid multiplication of formerly scarce animals that they become commonplace and cannot be sold for a large premium. In the mid-1970's, propagation by embryo transfer of several breeds of cattle that were rare in some countries resulted in a "boom and bust" cycle, and many entrepreneurs suffered serious financial losses (*48*).

Embryo transfer technology is frequently used to obtain additional progeny from cows with certain kinds of infertility. In some cases, the infertility is corrected in the course of embryo recovery procedures (*21, 49*). This application could lead to propagation of genetically defective animals, but it is justified when valuable cows become infertile due to injury, disease, or senescence. Many infertile donors are in the latter category; their ovaries produce normal ova, but they are incapable of maintaining a pregnancy. Similar phenomena occur in a number of other aged mammals (*50*). Pregnancy rates are normal when morphologically normal embryos recovered from infertile donors are transferred to suitable recipients (*21, 49*). On the average, only a few normal embryos are recovered per infertile donor, however, which makes such reproduction very expensive. Nevertheless, on rare occasions results are spectacular. For example, we have obtained 30 pregnancies over a 15-month period from one infertile donor.

Hundreds of bovine embryos have been exported from Canada to Europe (*27*) and to other regions of the world too. Clearly, it costs much less to transport embryos than cattle. Although some exported embryos were frozen, most were not. Logistics are complicated when embryos are not frozen—for example, synchronizing estrous cycles of donors and recipients in widely different time zones can be confusing.

Theoretically, there is an important epigenetic consideration in the export and import of embryos. Frequently, animals die or are severely debilitated by infection with local pathogens when they are introduced into a different country. When embryos are transferred to indigenous recipients, however, the young receive appropriate passive immunity through the colostrum (first milk), and the developing immune system of the calf becomes specifically attuned to the pathogens in the environment. There may also be other physiological and behavioral adaptations that are facilitated when an animal is born in a particular environment rather than being placed there at an older age. Although artificial insemination usually is the method of choice for introducing new genetic material into indigenous cattle, it is slower than embryo transfer because three generations are required to produce an animal with seven-eighths of the genetic makeup of the new breed. On the other hand, embryo transfer is considerably slower than importing breeding adults, if they survive.

One limitation to the export of embryos is the potential for spreading disease. In all likelihood there is less potential than with movement of animals, but very little is known about this (*51*). Embryo transfer has been used successfully to introduce new genetic material into specific pathogen-free swine herds (*52*).

Testing for Mendelian recessive alleles. If a homozygous recessive individual is mated to a suspected carrier of a Mendelian recessive allele, and eight normal offspring result, the probability that the individual is not a carrier is 99.6 percent $[1 - (1/2)^8]$. Without superovulation and embryo transfer, it would take more than the average reproductive lifespan to prove that a cow was not a carrier of a particular undesirable trait. Furthermore, the eight normal progeny required for the proof would all be carriers and, therefore, undesirable for breeding purposes. With embryo transfer, comparable proof can often be obtained within a year. This is especially important when an outstanding cow is a daughter of a carrier.

Embryo transfer is also used to prove statistically that bulls are not carriers of undesirable traits (*53*). Without embryo transfer, proof can be very difficult to obtain because sexually mature females with certain homozygous recessive alleles are extremely rare. Examples are dwarfism and syndactyly (mule-foot). If a single homozygous female is available, however, she can be superovulated and her genes thus amplified for testing bulls. Furthermore, for many traits, diagnosis can be made at 60 days of gestation. The recipients can be slaughtered at that time to speed up the test without harming the rare, homozygous donor. Recently four of eight sons of a bull known to be a carrier of syndactyly were found to be carriers (*53*).

Future Technologies and Applications

In vitro fertilization. Several reports provide some evidence of in vitro fertilization of bovine ova (*54*), but no pregnancies have been reported from such procedures. In fact, production of young from oocytes fertilized in vitro has been reported in only four mammalian species: the rabbit, mouse, rat, and human (*55*). There probably have been hundreds of attempts at in vitro fertilization in human beings, and many were successful in terms of ovum penetration by sperm; however, continued normal embryonic development to term has rarely been documented (*9*, *56*).

Little work has been done with in vitro fertilization in cattle, partly because of the lack of economic incentive and research support and partly because of alternatives to in vitro fertilization. Oocytes can be taken from bovine donors, transferred to inseminated cows to be fertilized, and then recovered for evaluation. Normal embryos can then be transferred to recipients for gestation (*57*, *58*). This procedure is tedious, and success rates are low. For commercial application, it will be necessary to mature oocytes and capacitate sperm reliably, preferably in vitro. Few young have been produced from oocytes matured in vitro (*57*), possibly because such oocytes are usually abnormal. Capacitation, that is, modification of sperm in the female reproductive tract required for fertility, remains relatively unstudied in the bovine.

One application of in vitro fertilization will be to circumvent infertility. Probably a more important use, however, will be as a quality control test for semen, since current laboratory tests are not highly correlated with fertility (*59*). Another significant application may be to provide large numbers of embryos from oocytes recovered from ovaries at slaughterhouses. A final application would be to extend the use of rare, valuable semen. Currently, millions of sperm are required per insemination dose for acceptable conception rates, yet only one fertilizes the oocyte. With in vitro fertilization, this number could be reduced, possibly even to one, if sperm were injected directly into oocytes (*60*).

Sexing embryos. It is not yet possible to separate X- and Y-bearing bovine spermatozoa reliably, but the sex of embryos can be determined karyotypically. Cells can be obtained by biopsy of morulae or by cutting off the tip of an elongating blastocyst (*61*). Most embryos continue normal growth after biopsy, although pregnancy rates appear to be slightly lower than those with unsexed embryos. The bovine sex chromosomes are easily distinguished; thus, sex determination is highly accurate when suitable metaphase chromosomes are available for examination. Unfortunately, procedures are tedious and time-consuming and usable preparations are obtained from only about two-thirds of the embryos (*61*). Therefore, three categories of approximately equal numbers result: male, female, and unknown. Before this technology can be applied on a broad commercial scale, it must be made simple, fast, inexpensive, reliable, and nondamaging to embryos. A fringe benefit of karyotyping is detection of chromosomal abnormalities.

An alternative to karyotyping could be detection of the gene product of the Y chromosome, H-Y antigen (*62*). Sexing embryos will be replaced when it becomes possible to separate X- and Y-bearing sperm without damaging them, a feat that may, however, be many years away.

Commercial applications of sexing embryos are based on the following considerations. Because of artificial insemination, the number of bulls required for breeding is greatly reduced, and the value of the few very good bulls is greatly increased. Thus, in a very few instances male calves will be more valuable than female calves. In the great majority of cases, however, females are considerably more valuable than males. It is financially risky if one must count on at least five females from ten pregnancies for profitability; for example, the probability of seven or more offspring of one sex is 17 percent if the true sex ratio is 50:50 (*63*). In most cases, then, female embryos would be transferred and male embryos discarded, used for research purposes, or transferred to produce bulls for fattening (*64*). An advantage of sexing when inducing twinning is that one can ensure that a male and female fetus do not develop together, resulting in a sterile female (a freemartin).

Twinning. Nearly 80 percent of the cows in North America are beef cows whose sole function is to produce one calf each year. Approximately 70 percent of the nutrients consumed by each beef cow are for her own maintenance, while only about 30 percent are for growth and maintenance of the calf during pregnancy and lactation (*65*). The conversion of feed into meat would be more efficient if cows had twins even though there are more problems with twins, such as higher losses at birth (*40*, *66*). Currently, embryo transfer is the most effective method of inducing pregnancies of twins in cattle. Hormonally induced twinning often results in two embryos in one uterine horn, which leads to more abortion than when one fetus implants in each uterine horn (*67*).

Because of a shortage of calves to fatten for beef, twinning in cattle may become economically viable in Europe and Japan before it does in North America (*68*). Average prices for newborn dairy calves to be used for meat were less than $100 in North America in early 1980 (*69*); and embryo transfer costs per successful twinning would have to be considerably less than this to be profitable. However, lower costs of the technology and inexpensive embryos from unwanted frozen male embryos or from in vitro fertilization of oocytes from slaughterhouse ovaries may eventually lead to commercial twinning.

A special case of twinning is the production of identical twins by separating blastomeres at the two-cell stage or cutting morulae in half (*70*). This would lead theoretically to twice as many calves per embryo and eliminate the occurrence of freemartins from twinning without the need for sexing. When methods of producing identical twins (or triplets, or quadruplets) become more practical, the major use may be control of genetic variation in experiments.

Progeny from prepuberal calves. The ovary of the prepuberal calf can be stimulated by exogenous gonadotropins to produce viable ova long before the uterus is capable of maintaining pregnancy. Such ova can be transferred to postpuberal animals to produce calves, although with very low rates of success (*71*). This is extending reproductive life in the opposite direction from the cow with a senescent uterus. In general, genetic and phenotypic information about prepuberal calves is limited; therefore, using them as donors would only be profitable in special situations.

Progeny-testing females. It would seem that embryo transfer technology could be used routinely to progeny test females for traits like growth rate, carcass characteristics, and milk production. However, the time required to collect the information would increase the generation interval to such an extent that the added information would be of little value in a well-managed breeding program (*72*).

Benefits of Embryo Transfer Technology

Currently, 70 to 90 percent of the female calves must be kept as replacements in the national dairy herd for those removed by death, senescence, disease, injury, infertility, and so forth. If all replacements could be obtained from the top 10 percent of the herd, then genetic progress from this source would be between three and four times as rapid as with the current 70 percent level of selection intensity (*73*), and even more rapid with sex selection. With a good artificial insemination program, however, nearly all genetic progress comes from selection of sires, not dams, so that even tripling progress from selecting dams leads only to a net 10 to 15 percent increase in overall genetic progress (*72*). Thus, the current cost of embryo transfer may be much higher than the return in terms of increased milk and meat production (*74*). Embryo transfer might be relatively more useful for beef cattle because selection of males is not as overwhelming a driving force in genetic improvement as it is with dairy cattle.

The industry should not rely too heavily on genetic and economic analyses of production traits. Farmers have used embryo transfer technology almost exclusively for purebred animals, and the relation between market value of purebred cattle and genetic value is low, although usually positive. Factors such as scarcity and economic health of the livestock industry have much greater impacts on market value of high-priced animals than does their genetic value in terms of production traits. Thus, cattle breeders use potential profit rather than some other measure of genetic value to determine if and when embryo transfer should be used.

The genetic value of dairy cows produced by embryo transfer was determined from data provided by the National Cooperative Dairy Herd Improvement Program in the United States. The 50 progeny with records in early 1979 (resulting from embryos transferred before 1976) were at the 18th percentile from the top for genetic value for milk production. Donors on file to date ($N = 275$) were about at the 25th percentile (*75*). Thus, dairy cattle donors selected for embryo transfer and the resulting progeny have been considerably above average in genetic value for milk production, even though there was also selection for traits such as body conformation, longevity, and popular blood lines. Of interest is that sires of embryo transfer progeny were approximately at the second percentile in genetic value for milk production of all bulls born.

It is too early to assess the long-term impact of bovine embryo transfer technology on the rate of genetic improvement nationally. Because of the relative ease of amplifying male reproduction, most of the genetic influence of embryo transfer in the near future will be through males produced by this process, or born to females as a result of embryo transfer. Although the cost and complexity of embryo transfer probably will limit its application to less than 1 percent of reproduction in cattle for the next decade, certain specialized uses will have far-reaching effects. For example, the genetic base of certain breeds rare in North America was quickly broadened by embryo transfer (*76*).

Analogously to putting eggs from whooping cranes into nests of sandhill cranes, embryo transfer can be used to rescue rare breeds or species from extinction. An example is the current effort to rescue the Angora breed of sheep from extinction in Australia. The breed is being reestablished from only a few animals by transferring resulting embryos into other breeds of sheep. The same technique might be used with endangered species such as the black-footed ferret and mountain bison. Females of closely related species must be available as recipients; if recipients of the rare or endangered species must be used, embryo transfer is not indicated because amplified reproduction of donors is usually at the expense of decreased reproduction of recipients.

It should be emphasized that embryo transfer is extremely useful experimentally, especially for distinguishing effects of the uterus from those of the embryo (*77*). An example is probing the causes of declining reproductive function as females age by transferring ova from young to young, young to old, old to old, and old to young uteri (*50*). Another illustration of the power of these techniques is that one could make identical twin female embryos, freeze one of them, and transfer it to the other when she becomes sexually mature so that a female gives birth to her identical twin sister.

Conclusions

Commerical bovine embryo transfer has developed into a $20-million-a-year industry in less than a decade. Currently reproduction of fewer than 5000 cows in North America (<1 in 15,000) is being increased in this manner, although use of embryo transfer technology is increasing rapidly. Probably 20,000 calves will be produced by embryo transfer in 1980, and their genes or those of their sons will be greatly amplified by artificial insemination.

Techniques will continue to be simplified and efficacy will increase, especially for freezing embryos; consequently, costs will decline drastically. It will probably be decades, however, before embryo transfer will be used on a scale comparable to artificial insemination. Techniques of genetic engineering, which require in vitro manipulation of ova, will require embryo transfer for application.

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Mr. Gore. Thank you very much.

Do you have the film fixed now? I would kind of like to see it. Maybe we can work that out and we can see it at another time after the hearing is over with.

Do you agree, Dr. Seidel, with the point made by Dr. Hunter that some version of cloning or replicating embryos will be necessary if these genetic manipulation techniques are to ever be economical?

Dr. Seidel. Yes. I think he made a very important point. Let me amplify just a little bit.

If one introduces these genes into the one-cell stage, they automatically get into every cell in the body of the animal that develops. So, that is the absolute ideal time to modify the genetic composition of an animal because the system takes care of the job by itself, getting in all the cells of the body.

It is absolutely necessary to have these techniques of embryo transfer—they are rather mundane even at the moment, but it is absolutely necessary to be able to keep the embryo in a test tube, to be able to inject things into it that enable it to survive, and then be able to put it back into an animal and have it survive to term.

Mr. Gore. It is also necessary to make it economical to be able to replicate the result of that activity, right?

Dr. Seidel. Yes.

Mr. Gore. Now, what is our capability now of replicating cattle embryos?

Dr. Seidel. I didn't provide any data, but let me try to summarize that.

I suppose there are two parts to the question. One, if you have a valuable cow, can you treat her with fertility drugs so she produces more embryos and that sort of thing. The answer is yes. On the average it is possible to get 10 or 12——

Mr. Gore. No, no.

Dr. Seidel. You are interested in the embryo itself?

Mr. Gore. The embryo itself. Well, let me start in a different place: cloning. I want to get a good accurate definition of cloning from the three of you.

First of all, something we can't do is we cannot take a full grown animal and take a cell nucleus from somewhere in that animal and put it into an egg cell and have an embryo develop according to the blueprint provided by the genes of that full grown animal? We can't do that yet?

Dr. Seidel. That is right.

Mr. Gore. The reason we can't do that is that by the time an animal is full grown, the cells have specialized to the point that most of the genes have clicked off and will no longer express traits. Is that correct?

Dr. Seidel. That is correct. However, it is entirely possible that as we understand how life works, how it evolves, that we will be able to dedifferentiate——

Mr. Gore. To click them back on again?

Dr. Seidel. Yes, that is correct. We don't know if that is going to be possible, but thus far it hasn't been possible, even in fish or frogs or whatever.

Mr. GORE. Right. Now, at this point—by that I mean the clicking off of the genes as the cells differentiate and specialize—that is the principal or the biggest obstacle to the cloning of an adult animal?

Dr. SEIDEL. That is correct.

Mr. GORE. Now, if the science of cell activation or gene activation moves more quickly than we now suppose it will, that obstacle might well be removed.

Dr. SEIDEL. It is possible, or it could turn out that there are irreversible changes.

Mr. GORE. We just don't know?

Dr. SEIDEL. Yes, we don't know. That is a problem in basic biology. There may be ways to circumvent the problem. For example, we could take Dr. Hunter's idea of an ovary that has 100,000 oocytes in it and we might freeze the 100,000 oocytes—let me change the direction slightly.

What we might do is make a cell line from one of those oocytes that in a way make it even cancerous, so it develops in a test tube indefinitely, and then use one of those cells to make an adult and see how good she is, and then make all of the copies we want.

Mr. GORE. Yes.

Dr. SEIDEL. So there may be that scheme of circumventing this.

Mr. GORE. I understand. I raise cattle. I have a sense of what the economics are in the industry and the principal breakpoint in terms of the economic impact of this science is when it can eliminate the uncertainty associated with trait selection.

In other words, now you can have the grand champion bull and the grand champion cow and it still is a terrific gamble as to what the characteristics of the offspring are going to be.

Whenever your science gets to the point where a farmer can choose with some certainty excellent characteristics of the offspring, then you are making an enormous impact on the industry.

Now, that is why the possibility of cloning an adult is such a potent possibility, because one could simply go to the Denver show and pick out the grand champion and order 10,000 identical to it.

The farmers in my district that spend a lifetime of selectively breeding marginal improvements into the gene pool, would soon be out of business, if they couldn't afford laboratories, et cetera.

Now, what you are saying is even if you can't do that, clone an adult, you might get to the same point if you could replicate or clone lots of copies of an embryo, freeze all but one, let the one grow until you could ascertain the genetic characteristics, take it to the Futurity or wherever, and if it is an excellent animal, then you have got 1,000 more in the refrigerator or in the freezer.

By that combination of techniques, you could have almost the same impact on the livestock industry. Is that what you are saying?

Dr. SEIDEL. That is what I am saying. We in fact can already do that on a small scale, by making identical twins or triplets or quadruplets. That is a small scale, but nevertheless it is nice to have a spare copy in the freezer if you have a real valuable animal.

Just to illustrate the power of the techniques, one might take an embryo and cut it in half and freeze the one half, transfer the other, and if it grows up to be a female, we can then transfer her identical twin sister to her so a female gives birth to her identical twin sister, which is a very powerful genetic tool.

Mr. GORE. You seem to take some satisfaction from these experiments.

Let me ask you this. Are there any foreseeable obstacles to the scenario that I just sketched?

Dr. SEIDEL. Actually Dr. Hunter is working on it specifically, so maybe he would be the one to ask.

Mr. GORE. Dr. Hunter?

Dr. HUNTER. No, I think you have expressed what we hope to do, to use the freezing methodology to preserve something that we consider valuable, until it is tested out in the field, and then retrieve it.

This is basically the same scenario that was followed in the early days of artificial insemination and is currently followed today, to select the superior animals.

Mr. GORE. Yes, but again, with artificial insemination you still have enormous guesswork and chance, you are narrowing the odds, but here you are eliminating the odds.

Dr. HUNTER. Right, but with young bulls, you collect semen from them, you then freeze the semen, you use it, you get your records and then you decide whether those bulls become the sires of the future or they go into meat production.

Mr. GORE. I well remember when the first shipment of dry ice arrived at our farm for that very purpose.

Now, what limit is there to the number of copies you can make of an embryo; in other words, you can divide it in half and you have two. Can you take each one of those two and divide those in half?

Dr. HUNTER. I don't think we know what the end point is. I would guess with today's science we are maybe talking eight.

Mr. GORE. Eight?

Dr. HUNTER. George may agree or maybe go a little bit higher, but I think in the not too near distant future we will probably be talking around 128.

Mr. GORE. 128, or more?

Dr. HUNTER. Or whatever the multiple is, yes.

Mr. GORE. It is important because the economic impact——

Dr. HUNTER. The reason I made that statement is because with the first mice that were born with Karl Illmensee's experiment that was done in Switzerland and published I think in 1981, he took an embryo that had differentiated into the three germ layers—in other words, it is down the road quite a ways—he took nuclei out of each of those germ layers and was able to get at least with two of the germ layers, live offspring.

There would be somewhere between 128 and 256 cells in that type of embryo. Those would be potential offspring.

Mr. GORE. Now, is it likely that the number of identical copies of an embryo that we are able to make will increase as the science progresses? There are not any theoretical obstacles, are there?

Dr. SEIDEL. If I could interrupt, I think it will be possible to make as many as you want.

Mr. GORE. As many as you want?

Dr. SEIDEL. Yes. I don't think there is a problem.

Mr. **Gore**. There is no theoretical obstacle?

Dr. **Seidel**. I don't think so, but one has to use various tricks and we are extending technology that really isn't there.

Mr. **Gore**. You are talking about the technology of micromanipulation?

Dr. **Seidel**. Yes. Well, for example what you might do is let the embryo develop—let's say at the moment there is a theoretical limitation of 100. Then you might take one of those nuclei, put it into another one cell, and let it go up to 100 again, and you could just keep repeating that indefinitely.

We are making it sound much easier than it is. There is a lot of work——

Mr. **Gore**. There is a lot of work to be done before that, but we are talking about the kind of work that could be done in this decade.

Dr. **Seidel**. We already know that in fact there isn't a theoretical limit of that sort of thing. That is different again from cloning an adult, to which there may be a theoretical limit.

One other point. We talk about finding the best animal we can and making all the copies. I really think we should be looking beyond that. We ought to be able to put something together that is better than the best.

Mr. **Gore**. To make a lot of copies of that?

Dr. **Seidel**. Yes, and then make something better than that, even. In fact, there is no one best animal, it depends on the environment. Like the best animal in Tennessee is not the best animal in eastern Colorado and it is not the best one for Canada. There are thousands of best animals, depending on the environment.

Mr. **Gore**. One of the subcommittee's motivations in examining this question—and I am going to yield to my colleague in just one second—is to try to ascertain the likely economic impact on a whole way of life that now exists among livestock breeders.

If it is likely that within this decade we will be able to make not just twins or quadruplets but hundreds of copies of an embryo and freeze, and further if it is likely we will be able to introduce desired traits into an embryo and then make copies of that, then why isn't it likely that the economic impact of this technology is going to be awesome for all of those, the livestock farmers, who do business as they currently do business? In fact that is likely, isn't it?

Dr. **Seidel**. Maybe. I just saw a cartoon the other day that was a pun on our twinning work where the rancher said, "I am already losing $10 a calf and now I will lose $20." I think it is important to note that these sorts of technologies historically have not improved the financial position of the farmer. Where the impact has been the lower food cost in the end because of the competitive nature of this society and so on.

Mr. **Gore**. I am not speculating on a positive economic impact for small livestock farmers. As their representatives in the Congress, in this age of unprecedented change, one of the things we ought to do is to try to make a better effort to forecast what is coming down the pike.

It looks to me like this technology has some pretty awesome implications for the livestock industry as it is now made up, both good and bad, but for the small operators some potentially bad implications.

Dr. **Seidel**. There are also opportunities, of course, if he can buy a frozen—if there are 100,000 copies of a good one and he can have one, too. Maybe these need to be patented or licensed or something.

Mr. **Gore**. Yes?

Dr. **Hunter**. I think the small operator, at least in Minnesota, is aware that these things are coming. Over Christmas break at school I went out and gave six or seven farm talks. I asked my audience, How many of you are using artificial insemination? Quite a few raised their hands.

How many of you are using embryo transfer methodology? I was amazed at the number of people that raised their hands. Maybe 20 percent of the audience. I said, How many of you are using frozen embryos? That, in December of this year, was pretty close to the forefront. There were several that were using frozen embryos.

I was talking about cloning. Naturally that is not out on the farm now, but the small operator, at least in Minnesota in the select group of breeders that I talked to, is aware that these things are coming and wants to use these technologies to, we will say, make more profit for the amount of time they have to put in.

Mr. **Gore**. Yes. Well, I hope the results are good, but we will see. Mr. Walker?

Mr. **Walker**. Thank you, Mr. Chairman.

Mr. Chairman, first of all, could I ask unanimous consent that some opening remarks that I have here be included in the record at the opening?

Mr. **Gore**. Absolutely. We already did that.

Mr. **Walker**. Thank you.

I must say that I find what you are having to say here fascinating; a little frightening, too, when you consider some of the ramifications of it that might be down the pike.

For example, it seems to me that you could well end up with the situation where the Kentucky Derby would be a test not of the various trainers involved, but of the guy that was the scientist who was doing all the embryo transfers and which farm hired the best scientist.

It appears to me, from what you are having to say here, that we are very close to that being the case right now, that we could get to that point very quickly, the very thing that makes this a potential economic advantage, given the fact that you could replicate embryos. It also, it seems to me, carries with it some danger as well.

Do we stand a chance somewhere along the line that if it became the technique accepted in the industry, that we lose significantly from the gene pool that is available to the country?

Dr. **Seidel**. Would you like a comment on that? It depends if we use our heads or not. In fact, it offers opportunities to do the exact opposite. We had been losing a lot of genes through artificial insemination and so on because instead of really millions of bulls there are only thousands involved in milk production with Holsteins.

What we can do is freeze embryos from whatever strains we want and store the genetic information for a few cents a month indefinitely for some centuries. So, if we do nothing and are not careful about it, we can in fact narrow the genetic base, but we can also use the techniques to do the exact opposite, to broaden it.

Mr. **Walker**. But the thing that makes it an economic advantage is to narrow rather than broaden; isn't that the case?

Dr. **Seidel**. In many situations, but again, as I am saying, it will cost very little to store this genetic material.

Mr. **Walker**. Yes, understandably, but the fact is that what you would have ended up producing and the lines that would be out there would be from a fairly narrow genetic base in order to make it economically viable. Isn't that a reasonable assumption?

Dr. **Seidel**. You want to use the best ones, and by definition you are getting rid of the less desirable genetic lines.

Mr. **Walker**. My question is, then, If we didn't use our heads—and we have shown ourselves perfectly capable in science areas over the years not to be able to use our heads very well—if we did not and narrowed the genetic pool, narrowed the lines considerably, don't we run the risk that something, maybe an unknown disease of some sort, could literally wipe out production for a period of time because it would apply to one line only and could virtually destroy much of the livestock production in the country?

Dr. **Seidel**. There is a cost to everything, a cost to any advance, and perhaps the best example is with hybrid corn. We are all aware of the fiasco about a decade ago where the male sterile cytoplasm did exactly what you are talking about and probably cost the U.S. agriculture industry $20 or $30 million because of that problem.

I say well, maybe we should be concerned about that, but we need to think about it in a broader sense. Each year hybrid corn is worth billons of dollars, and because there was a $20 or $30 million fiasco, that is one of the costs involved.

In a way it is a trivial cost compared to the benefits. Because of the knowledge of plant breeding and so on, we were able to solve that problem pretty quickly, in fact, effectively within a year.

Mr. **Walker**. I guess a question that would occur to a lot of my constituents beyond my farming constituents is how close are we to this technology applying to humans, or is the technology demonstrated here today applicable to humans but we just haven't been doing it?

Dr. **Seidel**. It clearly can be applied to humans, if that seems desirable, I guess.

Mr. **Gore**. It doesn't.

Mr. **Walker**. I mean that is the question. I think in many moral and ethical considerations it is something at least in this country that would be considered unacceptable, to apply the technology to humans.

The question becomes whether or not there are people with different ethical, moral standards that might desire at some point to apply it to human beings. So the answer to the question is really yes, that the techniques you demonstrated here today would be available to be applied by someone that was less concerned about some of the issues that plague us.

Dr. **Seidel**. You need a lot of cooperation from a lot of people to make them work, however. I guess you could imprison the women and transfer embryos to them or something. I am frequently asked that question, and I like to use the following example:

Dr. Anderson rightly pointed out that the most powerful genetic technique that we have today is artificial insemination. It is more powerful than embryo transfer for a variety of reasons: It is very easy to apply, it is not very expensive.

If we want to change the genetic composition of cows in Florida, the quickest way and cheapest way of doing it is with artificial insemination. If we want to change the size of men in New York City, we already have the technology to do that very, very effectively.

This just adds to that. Society, and I guess all of us, have a responsibility to see that these techniques are not misused. With every technique there is a possibility for misuse. In fact, the thing that comes to mind as the most lethal mechanical device in North America is the automobile. More people are killed with automobiles than anything else.

That is a cost of having automobiles. Similarly, there are costs to all these things. It is important to be aware of them.

Mr. WALKER. One of the interesting things about the automobile, though, is that we really didn't, as it expanded into our lifestyle and became a predominant part of our lifestyle, even consider many of the moral and ethical issues connected with the kind of easy transportation that it involved and all of the rest of the factors, all the way up to drunken driving and so on, that have had a major impact on our society. We didn't make many determinations about those. We let it grow and then all of a sudden found that we were confronted with issues.

I think that discipline is so important to the future of the world and human beings, that it is something where we don't want to be in a position of blindly going into something where we haven't at least tried to think through all of the moral and ethical issues.

Thank you, Mr. Chairman.

Mr. GORE. Let me say with respect to the application of these techniques to human beings, we have a series of hearings in the fall on the first of such questions that have presented themselves.

As far as the sciences concerned, I am told by the scientists that in many ways human cells, or the cells in question, are less complicated than bovine cells. Is that correct?

Dr. SEIDEL. It is.

Mr. GORE. So from a scientific point of view it is even easier with human beings, but of course the ethical, legal, and religious misgivings that many of us have, and that our society as a whole has, would come into play, and should, in my opinion.

I certainly want to associate myself with my colleague's remarks on how we need to do a better job of forecasting some of these problems before they express themselves in terms of, to use the automobile example, 50,000 deaths a year.

We have a vote on the floor. It is an important vote. I would like to ask some more questions, with your indulgence.

We will come back in 10 minutes.

[Recess.]

Mr. GORE. The subcommittee will come back to order. I apologize for the delay.

Let me ask the three of you, How close are we to modeling the characteristics of an animal from the information screened from the embryo? In other words, is that totally out of the picture or is there any work being done on that at all? In other words, what I am getting at is the ability to forecast the traits expressed by genes that are identified.

Dr. SEIDEL. Let me use sort of a trivial example that has great impact. We can look at the embryo and determine the sex. That is a pretty important outcome. So in that sense Dr. Anderson's work is already going to have a big impact, and maybe he would want to say more about it.

Mr. GORE. Dr. Anderson?

Dr. ANDERSON. I won't add anything more to what Dr. Seidel said about determination of sex other than that several years ago it couldn't be done and it can be done reliably now. So, it would be shortsighted to think that we can't move into other areas.

Mr. GORE. That is not a nonintrusive technique.

Dr. ANDERSON. The technique that we are working on uses an antibody against a male factor. The previous technique, where embryonic membranes were karyotypes, a very large embryo was used. The success after transfer was low, and successful sexing was not guaranteed. The technique that we have is about 85 percent accurate and strictly on laboratory animals at this time.

Mr. GORE. But there is no way to predict other characteristics?

Dr. ANDERSON. No, not in terms of traits that at least I, as an animal scientist, feel are important; that is, the overall efficiency of production of the animal, which determines its genetic merit in the largest sense. We cannot predict other characteristics because, as I mentioned before, we don't know how those traits are inherited.

There are a few examples of major genes. For example, one exists in sheep; we know that if the gene is present ovulation rate increases. If that gene is not present, the animal has a low ovulation rate. We know the gene exists. We do not know how it is inherited, which chromosome it is on, what other traits are associated with it or all the physiological effects of that gene being present.

So, our knowledge of genes in domestic animals is very narrow at this point.

Dr. HUNTER. Perhaps along the same line, in terms of milk production, by giving an animal injections of a growth hormone you increase the amount of milk that that animal gives significantly. By significantly, some of the reports I have seen have said a 40-percent increase in milk production by a daily injection of a bovine growth hormone. A number of the genetic engineering companies are pursuing those lines.

But coming to the embryo work here, I believe it was Peter Hoppe's group that took some dwarf mice and inserted the gene for growth hormone into them, and some of those mice took the gene and developed not as dwarfs, but as normal sized mice.

Now you can take it a step further—and no one has done this with cattle—if you could isolate the gene that controlled the growth hormone in cattle, insert it into an embryo, you might have an animal that would produce hyperamounts of growth hormone and produce more milk than it is "genetically capable" of producing.

So all of these things are coming very quickly.

Mr. GORE. How close are we to that sort of thing? This decade?

Dr. HUNTER. I would say probably within 10 years.

Mr. GORE. Within 10 years?

Dr. HUNTER. Within 10 years, and I say that because they have put the gene into mice. The problem, as I see it, with cattle would be identifying the gene so that you could cut it out and insert it into some type of a replicating system. I express my ignorance. I don't know if anyone has characterized on which chromosome the gene for growth hormone is present in cattle

Dr. ANDERSON. Or any other species.

Mr. GORE. Dr. Anderson says they have not.

Dr. ANDERSON. I would like to point out—not to get into an argument with Dr. Hunter—that work with growth hormone has been over extremely short periods of time. There is no indication that inserting a gene for growth hormone is going to have any positive effect on the ultimate growth or milk production in an animal.

Transfer of the gene for growth hormone may have beneficial effects, but it is not realistic to think that simply because growth hormones affect growth, if we put its gene into an animal a positive effect on growth will be observed.

Mr. GORE. So there is some substantial uncertainty?

Dr. ANDERSON. A lot of basic research needs to be done on the mechanism of action of those hormones before we can predict the effect of their transfer.

Mr. GORE. Are we facing constraints in the manpower available for this sort of research work?

Dr. HUNTER. I don't think we are being held back by lack of manpower. I think we are being held back by lack of money to do the research. When we work with large animals, it is extremely costly. We could do all our work with rats and mice, we could do some good research, and perhaps it is applicable to rats and mice, but the ultimate test is how do cattle respond under the same conditions.

The economics of how much it costs to keep a cow in the laboratory adds up. In our setup, we could be charged $4 a day per cow. When I get embryos, it costs me $300 per cow that has ovulated to get the embryos. My research budget is used up very quickly just maintaining animals.

Mr. GORE. If I was in the livestock business in a big way, why wouldn't I plan on assemblyline production of embryos, freezing them, monitoring the characteristics of the sets, and shifting production into that kind of operation?

What I am getting at is how likely is it that the industry will operate in that fashion in the foreseeable future, in this century?

Dr. SEIDEL. First the technology is much too expensive if you are going to eat the end product. Where it works is if you can produce an animal that is ideal and then spread those genes by artificial insemination, for example. That is already economically feasible and is being used.

So, if you can amplify what you do—that is, make breeding stock—it is already there for some things, but in terms of assemblyline, to eat it in the end, with the profit margins and so on for feeding cattle it is much too expensive. That is why these expensive techniques wouldn't work for that application.

Mr. Gore. That is why I said earlier that the breakpoint for the economics of this thing is how many replications of the same embryo you can make and how much it costs. That is the key stage, isn't it?

Let's assume that you went from 4, 8, or 16 up to 1,000. Presumably the cost of each increment would not be that much, but maybe that is an unreasonable assumption.

Dr. Seidel. Again, it depends how you get from point A to point B. For example, if a carcass is worth $600—and that is maybe not out of line for a beef carcass—you certainly can't invest more the $600 in producing it, no matter what you do.

Right at the moment just the embryo transfer work under most circumstances, even the most optimistic, cost several hundred dollars. So even if you had the perfect embryo, it would still cost you some hundreds of dollars to do something with it.

There may be ways of getting those costs way down, however, but right at the moment they are pretty high.

Mr. Gore. So the impact is likely to be limited for the foreseeable future to the herds that are bred for the gene pool?

Dr. Seidel. Yes.

Dr. Hunter. One other thing I might point out on the cost, about 3 or 4 years ago the cost for an embryo transfer was in the neighborhood of $4,000. Today the cost may be $1,000 but it is probably closer to $600. That is in a relatively short period of time.

Methodology has evolved that quickly to go from a surgical technique, which is very expensive, to a nonsurgical flushing of the embryos from the uterus, which means all of a sudden it comes closer to being practical.

Mr. Gore. Anything that any of you would like to add?

Dr. Seidel. I guess there may be a possibility of seeing the last 2 minutes of the movie, if anybody is interested.

Mr. Gore. I would like to see it, sure.

[Movie shown.]

Dr. Seidel. Here is actually dividing the embryo into two parts with the microsurgical blade. Next is removing one of the halves with a micropipette and making sure the original half stays in.

We have done several hundred of these. The technique works very well, actually.

This illustrates putting half an embryo into the surrogate zona pellucida, the one that was taken from the unfertilized ovum. There we have the two good embryos made from a good one and a bad one. Those are in a sense cloned and will develop into identical twins in many cases.

There is a little bit of a sequence here on making a chimera by injecting one cell into another. This is with the mouse again. If this were from a black strain of mouse and this a white strain, this embryo may then develop with a spot on it. So that just shows how that is done. That is one method other than the method that Dr. Anderson described. There are some ways of cloning animals using that technique as well.

There are about 30 seconds of movie left, I believe.

This is a sperm head. This happens to be a mouse sperm. It is injected into the ovum. I call this fertilization by brute force; that is, actually injecting the sperm into the ovum, so one only needs one sperm for the fertilization rather than a large number.

Thank you.

Mr. GORE. Before going on, let me ask you a question that I started to ask early on; that is, for a precise definition of cloning.

Dr. SEIDEL. I have a slide that I use. It has about seven definitions of cloning. It comes from the Greek work klon, k-l-o-n, which means to slip a plant.

Mr. GORE. To what?

Dr. SEIDEL. To slip a plant; that is, if you take a leaf from a plant and put it into the ground you get a new plant that is just like the parent plant. That is the origin of the term. So in a broad sense it is a sexual reproduction, reproducing without fertilization and merosis, the other part of sexual reproduction. So it is in a way making a copy without going through the biological aspects of sex.

A very narrow definition of cloning might be nuclear transplanting; that is, transplanting a nucleus from one organism into another so that the second has the genetic components of the first. Then there are a whole lot of definitions in between, so you can take the word and use it in a number of of senses.

Mr. GORE. Dr. Hunter?

Dr. HUNTER. I would agree. I cannot expand upon what George has said.

Dr. ANDERSON. I refer to cloning as reproducing a genetic copy.

Mr. GORE. Will the small farmer ever be able to get into this sort of operation, or is it reasonable to conclude that the capital investment is likely to remain so immense that it will be the exclusive province of rather large corporations?

Dr. HUNTER. I think the small farmers are going to be into it, just as the small farmer is into artificial insemination today. The small farmer has the use of the best bulls available in the United States at a reasonable cost today, and I see in the near future, with embryo transfer and the subsequent cloning process, he will have or she will have at their disposal these superior embryos to hopefully make more profit.

Mr. GORE. I hope you are right. Anybody disagree with that?

Dr. SEIDEL. I consider my father a small farmer. He makes his living by milking cows. He has 50 cows. He has been able to exploit some of this technology for a profit.

Mr. GORE. He probably has a good consultant at reasonable rates. Let me thank you, all three, very much for your help.

Mr. GORE. Our second panel consists of Dr. Robert Zimbelman, research manager for experimental agricultural sciences with Upjohn in Kalamazoo, Mich., and Dr. Autar Karihaloo, a director for embryo transfer with Carnation Genetics in Hughson, Calif.

Gentlemen, welcome to both of you. As with the previous panel, without objection, we will include your entire prepared remarks in the hearing record and invite you to proceed.

Dr. Zimbelman, if you want to start off, we are delighted to have you and invite you to proceed.

STATEMENTS OF DR. ROBERT ZIMBELMAN, RESEARCH MANAGER, EXPERIMENTAL AGRICULTURAL SCIENCES, THE UPJOHN CO., KALAMAZOO, MICH., AND DR. AUTAR K. KARIHALOO, DIRECTOR, EMBRYO TRANSFER AND RESEARCH, CARNATION GENETICS, HUGHSON, CALIF.

Dr. ZIMBELMAN. Thank you for this opportunity to participate in your hearings.

My perspective is that of an animal scientist with a research history in reproductive physiology. My training in genetics was at a time when genes were a mystery in a way that no longer is true today.

Others on your panel are more up to date on the specific technology of genetic manipulation, but I have had many years of experience in trying to introduce new technologies into animal agriculture related to control of reproductive processes. It is then from this latter perspective that I present my views.

I have attempted to categorize my comments according to the following areas: First, business considerations; second, technical considerations; third, bioethical considerations; and fourth, comparative aspects of animal genetic engineering versus plant or microbial.

Mr. Chairman, I would like to give you my summary first so that we may get that on the record, further I will try to at least get through the business and technical considerations, so that if you feel we have to stop because of time, then we could leave the others to the written comments. Also if you know where I am going, you can judge as I testify, whether or not I am supporting that summary.

My summary concerning the role of various technologies involved in animal genetic engineering leads me to the following conclusions:

Technologies involved in animal genetic engineering involve those of artificial insemination, embryo transfer, estrus synchronization, cell fusion, and insertion of specific genes, or DNA, into animal cells, ova, or embryos.

Various or all of these technologies would be utilized to varying degrees in any given program to improve the genetics of animals related to disease resistance or production performance.

Artificial insemination has been widely used in mass application for many years and should continue to be valuable. Embryo transfer and estrus synchronization are technologies now available in some species, but only for limited application with specific breeding situations and valuable animals at the current time. Progress is needed in animal management and the economics of these technologies before widespread adoption occurs in cattle and other species.

Recombinant DNA techniques are being studied but are not yet clearly enough understood to be able to assess their full impact on either a short- or long-term basis. The timeframe for the ultimate genetic engineering—namely, transfer of specific DNA or genes—is too long term to be predictable with any assurance.

The impact of such technologies will be significant if the 10- to 20-percent improvements in productivity were achievable with any wave of application of these technologies. It appears unlikely that the biological system of the animals would be impacted thorugh a single application of the technology to the extent of the 100- to 200-percent improvements.

Biological systems have been highly developed through periods of evolution and are maintained with a delicate sense of balance. Any major changes in those systems would be, I believe, achieved only through small changes at a time.

Now I will go back and cover the business aspects.

BUSINESS ASPECTS

Improvements in genetics of farm animals have come throughout our history mainly through the efforts of individual breeders and university personnel who did research in population genetics more so than through industrial firms getting involved in mammalian genetics. The one exception to that being the use of artificial insemination, which has had the impact of making available superior sires to a much larger base of females with which selected matings could be made. Associated with that impact has been increased efforts by those artificial insemination firms to identify the truly superior sires with which to make genetic progress.

The major industries involved other than the livestock industry itself have been the feed industry and the animal drug industry in impacting on animal agriculture. Obviously both the feed industry and the animal drug industry have a stake in the genetic makeup of the animals to be fed or treated and how this might change in the future.

Potential alteration of the genetic basis of food-producing animals could influence their response to either required nutrients or their efficiency of utilization of nutrients or their susceptibility to diseases or agents which improve their yield of food.

Therefore, industries not now in the animal genetics business need to think of the impact of these developments in two ways: One, as a new venture for their business which might be a profitable means of being involved in furnishing animal products to the world in the future, and two, as a defensive measure to survival of their business.

If animal disease resistance, for example, can be either engineered into the animal or induced through new approaches to vaccines, then treatments, either prophylactic or therapeutic, may not be needed in the future as they are today. This would mean that the companies that provide those treatments would not be needed.

Agents which improve performance may be doing so through mechanisms which could be engineered into the animal so that drugs which stimulate those increased efficiencies might not be necessary.

These seem to be the major business decision frameworks which a company like the one I represent needs to consider as new approaches to animal genetics. Proper consideration of that potential must be taken very seriously because it may relate to the actual survival of our business, so my comments are with that kind of serious consideration.

TECHNICAL ASPECTS OF TECHNOLOGIES INVOLVED

There is no question in my mind that artificial insemination has had a major impact on increasing the efficiency of production of animal products over the years.

That technology, approximately 40 years old, was a forerunner in genetic engineering of a sort and has been successful in mass application because it was relatively easy to perform collection and preservation of semen. The end result was relatively normal reproduction rates through procedures which were relatively simple and could be handled by persons with a reasonable amount of training and expertise.

The potential for embryo transfer has been much slower in implementation because, I believe, of the greater difficulty and greater expertise required by the technology. Therefore, it has been limited to a much narrower market; namely, that of very valuable animals.

Our company has been trying to get the concept of estrus synchronization incorporated in commercial beef and dairy herds for the last 20 years. It has been very difficult to get this new technology accepted, despite great advances in the ease with which synchronization can be achieved with acceptable conception rates.

I believe this means that even though certain advances in technology are available and can be achieved, the management of our livestock industry and the economics associated with the implementation are also extremely important. These factors need to be consistent with attempts to implement the technology in order to achieve widespread acceptance.

It appears to me that significant advances are occurring in embryo transfer but that it may be some time before cattle production systems will allow widespread application of this technology. Certainly some of the reports of injecting DNA into mammalian ova or embryos indicate a promise for genetic engineering approaches that are truly different from the past. Genes responsible for certain mammalian proteins have been isolated, identified, and it has been demonstrated that they can be inserted into the genetic material of other organisms than the one from which they were obtained.

All of this is truly remarkable and in some ways the science has progressed faster than even participants in the science would have predicted a few years ago. However, it appears that we must still go a long way before practical applications can occur.

Each month records considerable scientific progress in understanding DNA and being able to isolate DNA fragments responsible for certain key functions in organisms. However, each month also reveals surprises and significant blocks to progress that were not anticipated in our limited understanding of how genes act.

As an example not totally related to animal genetics, that came out during the previous question period, let me use bovine growth hormone. The gene for bovine growth hormone has been obtained from pituitary glands of cattle has been cloned, and copy DNA has been made of it and it is being worked on by several companies. Genes for the growth of animal species have been introduced into micro-organisms which have the potential to produce these animal hormones for administration to animals.

The potential for the pharmaceutical industry to take advantage of these kinds of developments seems relatively close, perhaps in the next 5 years. For livestock species, though, there are two continuing problems: One, the economics of administering such drugs. Even if the cost of the bulk drug is reasonable—and that is what most proponents keep mentioning—the drug delivery system must also be economical, and two, the practicality of drug delivery. Much of our livestock is not handled routinely, and even for those livestock which are handled routinely, farmers do not wish to make daily administrations of drugs by injections.

Orally active drugs can be administered routinely in the feed for animals which are fed but not for animals which are on the range. Proteins such as animal growth hormone and insulin, which have received a lot of attention, are generally not active orally and therefore systems for the administration of these drugs in a practical and economical fashion remain a significant challenge.

It seems to be a major leap to the level of incorporating those same growth hormone genes into the livestock species themselves, so as to eliminate the need to administer the appropriate hormones parenterally or through the feed.

There are many, many more aspects which we have not yet solved or perhaps even identified:

Can the gene be inserted in a way to be incorporated in the appropriate location to be expressed at the appropriate level by the animal?

If the gene is incorporated in the wrong place, will it interfere with other physiological functions; in particular, with reproduction? One line of mice resulting from the addition of human interferon genes to their genes has been reported to have sterility in the males.

Is the limiting factor for the animal to produce more growth hormone influenced by inserting an additional gene in the animal, or do we need to genetically engineer the controlling mechanisms in the brain which govern hormone production?

As an analogy, if your house is too cold because your thermostat is set at 62, it doesn't help to add a bigger furnace. You have to turn the thermostat up. I don't think anybody knows whether we should add a bigger furnace or change the thermostat.

One can go on with such a list of questions, which is almost endless and for which we do not now have information today. It may well turn out that we don't need to have answers to all those questions but that attempts to implement the technology may be successful. This situation has occurred in the past, but we cannot rely on that possibility.

It is my suspicion that before we have highly efficient food-producing animals with specifically engineered genetic capabilities beyond those which they now have from many years of selection and breeding, that we are going to find much basic information is still needed.

We will probably run into roadblocks which help us to define the kind of basic information on the physiology, biochemistry and cell biology which we need for understanding before we get to the level of engineering animals by recombinant DNA.

As was already said, very few desirable traits of animals are probably controlled by one or a few specific genes whose products can be easily identified. Many characteristics of health or performance are likely influenced by several genes, not necessarily located together in the genome.

Therefore, I believe a plan to fund research in these areas would be a very wise public policy. The research funds should be made available and assessments made of the progress and the identification of the areas to which these research funds should be channeled.

BIOETHICAL CONSIDERATIONS

Much of the progress made previously was from NIH or NSF for broad biological research, often with medical implications. It now appears that future progress will need to be funded by agricultural interests, such as the USDA.

Although there would always be concern about tampering with genetics of animals because of precedence and the nearness of those animal applications to humans, I do not believe there should be major concerns to limit potential applications as we currently understand the possibilities.

It seems a significant enough challenge to take naturally occurring genes and to attempt to introduce them into future strains of animals. It appears that the current proposals to do genetic engineering of animals do not involve anything that would have the potential for any undesirable kind of changes in those animals.

In addition, if my predictions are correct—namely, that we must have a much better understanding of the biological processes which we are trying to alter before they become altered—then we will have a much greater knowledge of what exactly has been achieved through these changes, and we will be able to assess their impact on food supply before implementing those changes.

PLANT AND MICROBIAL VERSUS ANIMAL GENETIC ENGINEERING

For comparative aspects of animal engineering versus plant or microbial genetic engineering, it seems to me that the progress in microbial genetic engineering comes about through being able to expose large numbers of cells to potential transformation and then to have selection mechanisms to determine which cells have been modified by incorporation of that gene.

The same potential exists for plant cells to be cultured in large numbers and to then select out through various mechanisms cells which have been altered to contain one or more gene that one desires to incorporate. The approaches to animal genetic engineering do not seem to allow this capability of large cell numbers as long as ova or embryo manipulation is the prime method.

Today, it appears that animal cells do not have the capability of mass cloning, as is available for microbial cells or plant cells. Somatic, or nongerm, cells of animals do not seem to have the property of regeneration, called totipotency, that exists in plant cells. Fusion with certain tumor cell lines seems to confer regenerating properties to animal cells. Much research is probably needed to control such procedures in a desirable fashion, however.

Certainly then the micro-injection of DNA into ova is much more limited in scope than the procedures available for plant or microbial cells. The effort to put new genetic material into large numbers of ova, then to expect many of those to fail, to evaluate those few which have been altered versus those which have not been altered, would seem to be a large enough task that one could predict major investment in funds before significant practical achievements will be made.

This may seem an overly pessimistic view of the situation, particularly for a person whose training is in animal reproductive physiology, but it is where my judgment comes out today.

I hope to have an open mind about it and to keep assessing scientific developments. I hope I am pleasantly surprised by the ease with which progress may be made, but I think only the future will tell and only investment of sound research dollars will allow us to make the final judgment.

Thank you.

Mr. GORE. Thank you very much. We will hold questions until the panel is completed.

Dr. Karihaloo, welcome. Please proceed.

Dr. KARIHALOO. Thank you, Mr. Chairman.

I apologize for my accent, but I will be slow and hopefully able to make myself understood.

Mr. GORE. No apology necessary.

Dr. KARIHALOO. I don't have to add much more than what was already discussed by the panel before us. However, from the commercial point of view, we still need a lot more basic work in the area of embryo transfer and related areas before we can go into more sophisticated technology of cloning or gene amplification and so forth.

Even today, although the technology of embryo transfer commercially has been going on for the last 10 or 15 years, still there are major problems which need to be solved to make it profitable to the common man and to the small farmer, who can afford it. The reason the prices are high is because success with this technology is very limited.

Genetic improvement of cattle can be divided into two steps: The identification of the most productive breed under the management and the geography of the particular operation, and subsequently the long-term implementation by selection of the best performing animal in the identified population.

In cattle, the progress that can be made in both steps of livestock improvement is limited by the low rate of female reproduction. Therefore, any increase in the reproductive performance of selection would contribute to the proliferation of the superior bloodlines and more intense selection.

During the seventies, a few commercial embryo transfer units were established in the United States on the basis of the economic demand to meet the first step by increasing the number of animals of imported breeds that had been limited in number.

More recently, however, emphasis of embryo transfer has been directed toward proliferation of offsprings from matings of genetically superior sires and dams to enhance selection intensity and expansion of superior gene pools.

Normally, a cow can produce four to five offsprings in a lifetime by natural reproduction. Therefore, the reproductive potential of a cow is small, as compared to that of a bull, but in terms of the number of immature eggs present in the ovaries of the young heifer, it is still quite prodigious.

For example, the ovaries contain approximately 250,000 immature eggs at the time of birth. Normally, only a tiny fraction of this vast number completes maturation, as one egg at a time is released in the course of each estrus cycle throughout the animal's life. The remainder of the egg population is never released, but throughout the life of the female, the egg number is continuously being reduced by degenerative processes.

The physiological mechanisms governing the selection of immature eggs for maturation and release are not yet fully understood. However, injection of various hormones may cause the maturation and release of more than one egg following heat in a cow. This phenomenon is termed "superovulation."

Release of about 100 eggs or more has been achieved, but the response of an individual cow to such hormonal treatment is extremely variable. On an average, the total number of normal embryos which are actually collected from superovulated cows is about six per flush.

Collection of embryos for transfer is performed nonsurgically about 6 to 8 days after the donor animal comes in heat. This procedure usually allows recovery of about 75 percent of the eggs shed by the ovaries. A relatively simple sterile solution of various salts, sugars and proteins is used for flushing the embryos from the genital tract of the donor cow for their transfer into the uteri of the recipient cows.

Only recipients which are in heat on the same day as the donor animal give maximum pregnancy rates after egg transfer. This entails considerable practical problems.

The introduction of prostaglandin and related drugs for induction of heat in cows has greatly facilitated the synchronization of the donor and prospective recipients, but it is impossible to predict how many recipients will be needed before collection of the embryos from individual donors.

At the present time, the practicality of embryo transfer is limited by the unpredictable superovulatory response of the donor. However, some interesting developments have occurred in the field of reserach on embryo transfer during the last few years which have somewhat changed the picture.

Particularly rewarding research has been carried out on embryo preservation. Cow embryos can now be cultured in test tubes for several days with relatively minimal loss of viability upon transfer.

However, the technique which holds the greatest promise is deep freezing of cow embryos. Although the results obtained in deep freezing of cow embryos are quite encouraging, this method has by no means reached the stage of perfection. Even so, it has in one stroke somewhat solved the major problems of planning transfer, long-term storage and long-distance transport of embryos.

The principles underlying embryo freezing are similar to those governing sperm freezing. Whereas a dose of semen contains many millions of sperm, of which many may be killed during freezing and thawing without affecting the efficiency of insemination, embryo freezing is a much more demanding process necessitating the survival not only of individual cells, but also the retention of their organization within an embryo.

Embryos which survive deep freezing can be recognized with a reasonable degree of accuracy by microscopic examination immediately after thawing. Therefore, the recipients do not have to be wasted on embryos with no chance of further development.

The majority of the deep frozen embryos transferred by Carnation Genetics have been stored in liquid nitrogen for 2 to 3 months.

Techniques for micromanipulation of embryos have reached a great degree of sophistication in laboratory animals. They have been extended into bovine species to produce chimeric calves, sexed calves and identical twins.

The ability to predetermine and preselect the sex of the newborn calf would be of enormous practical importance. To date, all efforts in separating X and Y chromosome-bearing spermatozoa have resulted in no significant alteration in the sex ratio of the live young.

It is reasonable to predict that in the immediate future, sexing of early embryos is likely to prove a more practical method of manipulating sex ratios.

Widespread use of embryo transfers in cows will be highly dependent on the level of efficiency eventually attained in solving the basic technical problems now encountered in embryo transfer. Nevertheless, major advances have been achieved within the last few years, and these technical problems are the ones most likely to be solved in the near future if the present research pressure is kept up.

Given acceptable efficiency in embryo transfer as such, the following factors would have profound impact on the cattle industry: Increase of the presexed calf crop from individual outstanding cows; the production of identical twins; possibility of rapid change in breeding aims within a herd; and export and import of cattle in the form of embryos.

Mr. Chairman, embryo transfer is clearly no substitute for conventional breeding at its best, but could become a formidable supplement. It is time this technology receives serious consideration by anyone concerned with cattle production.

Mr. GORE. Thank you very much.

Dr. Zimbelman, you seem to be the most cautious or pessimistic of our witnesses today. Let me ask you about the scenario that I explored with the first panel, the possibility of creating multiple clones of an embryo, freezing and preserving all but a representative individual, ascertaining the characteristics of the individual animal as it grows, and upon determination of its quality, then marketing of the replicas that were preserved, perhaps even selling them to distant farms in frozen form.

What is to prevent that scenario from becoming real?

Dr. ZIMBELMAN. It probably makes a difference whether you are talking about the beef industry or the dairy industry, but I guess I am pessimistic to a degree because of estrus synchronization for the beef business have a lot of farmers out there that try to get their cows through on fence posts and scenery, and when they try to synchronize estrus in their cows, they are not even cycling yet, because they are trying to save a few dollars on feed.

I just think they should be convinced to improve their efficiency of management by a few dollars in feed before they spend megadollars on a frozen embryo.

Now, that could change rapidly. I don't know. We hope to keep our eyes open if things change, but with the difficulty in getting producers to improve production through a few dollars in feed to better nourish animals, I guess I am not convinced of mass application—sure, breeders will, use on their very valuable animals, certain sophisticated technologies.

I think the technology is there, but I think cattle management and economics are things that we found are real deterrents to introducing some new technologies. What would make those change, I don't know.

Mr. **Gore**. Let's take the example of beef cattle and hypothesize an operation that is large enough to have several sets of cloned embryos. It allows one in each set to develop until the characteristics can be determined.

Let's further hypothesize that one of those individuals representing a set wins the Denver show and they have 100 more or 1,000 more identical to it frozen, as frozen embryos.

A farmer reads an advertisement in Livestock magazine saying for, I don't know, $1,000, $5,000, whatever, $10,000, he or she can purchase a frozen embryo that is identical to the winner of the Denver show.

Isn't that an economically viable hypothesis? I mean, why isn't that farmer going to be tempted to spend megadollars, as you say, or more money on a frozen embryo that is identical to the best animal of the year instead of improving feed efficiency?

Dr. **Zimbelman**. I am not sure that you and I are disagreeing, really. I think there may be such uses for very valuable animals—but how many of the cattle out there are really involved in being prepared for the Denver show? That is a select part of the population. I am talking about having an impact on all 40 million beef cows. I think it is something quite different than the kind of situation you are talking about.

Mr. **Gore**. Well, you have got different levels of the industry.

Dr. **Zimbelman**. Right.

Mr. **Gore**. Let's talk about the purebred. Let's take purebred Angus. That business is likely to be impacted by this in a big way, isn't it?

Dr. **Zimbelman**. Yes. I am not disagreeing with that, but if you are talking about the 40 million, including the beef cow, I think it is going to take quite a while for very sophisticated technology to get there.

Now, if you produce valuable breeding animals then you can use AI to get to the mass numbers of animals and that would probably be at a cost to most producers.

Mr. **Gore**. But they are just one step removed from the larger number that we eat.

Dr. **Zimbelman**. Sure.

Mr. GORE. I mean, it is all part of the same production system.

Dr. ZIMBELMAN. I think, though, my pessimism is more to the insertion of specific genes. I think if you followed my text carefully, I said estrus synchronization, and embryo transfers seem to be making a lot of progress and are now available for valuable animals, those could expand with the economics and management compatability.

Concerning the specific transfer of genes, I think we have a lot of questions, and I would hate to put a timeframe on it.

Mr. GORE. Do you want to comment on that, Dr. Karihaloo?

Dr. KARIHALOO. Yes, Mr. Chairman. There is no saying how much impact it will have on the industry, certainly a great deal of impact, but I don't think we have enough technology or there will be enough technology available even in another 50 years to have that superior animal identified at a cost which will be feasible to buy them since we can only clone, so to speak, the embryonic embryos and the rest have to be frozen.

It will be quite a while—if we had a mechanism whereby we could identify a superior animal before it is born, then of course it would have an impact.

Mr. GORE. Well, you see, in this scenario you have that because if you have a frozen embryo that is identical to another embryo that was not frozen but has been allowed to mature, in a very short period of time, in a short number of years, you can identify the genetic characteristics of the embryo that is still frozen.

Dr. KARIHALOO. That is true, Mr. Chairman, but by the same token, wouldn't it be easier and cheaper and more efficient if we could clone eggs from a fetus or from an embryonic ovary which we knew already carried the superior gene?

Mr. GORE. How are you going to know that it is an embryo?

Dr. KARIHALOO. That is the whole point. But the other way we will have to do thousands, maybe tens of thousands, to come up with one superior combination.

Mr. GORE. But that is still fewer than you have to do with selective breeding. Now obviously another way to get the same result would be to resolve the uncertainties surrounding the gene activation system and hurdle the obstacles to the cloning of an adult animal. That may or may not be possible, according to the best testimony we have been able to get.

It seems to me you do get to essentially the same result with the combination of cloning of embryos plus freezing and preservation. Do you disagree?

Dr. KARIHALOO. No, I certainly don't disagree, but it would be easier and cheaper and more feasible if we had another method. I don't know. Maybe there will be a method in another 50 years, who knows.

We know already that this offspring which is still in the fetal stage is indeed carrying a superior gene pool, and of course that technology is feasible. Then you can freeze the embryos carrying the gene pool, but otherwise it is going to be immense work for very little gain.

Mr. GORE. Well, I asked the earlier panel about the possibility of forecasting or modeling the characteristics of a grown animal from the information gleaned from the genetic makeup of the embryo.

Evidently there is very little work done on that, and the prospects of such forecasting, that is not an active field, evidently.

Dr. KARIHALOO. It is in the minds of various people. I mean, it is on the drawing board and people are certainly thinking how to go about it. It may not happen now, but I am sure in the future something in that direction may come up. Then we will have the tremendous impact of what you are saying.

Mr. GORE. Yes. If you could identify and catalog the correlation between specific genes and expressed traits and with the use of computers model the characteristics of an animal on the basis of the information gleaned from the embryo, then of course that would be another way to do it. That seems to be farther off, but who knows?

Dr. Zimbelman, I appreciate your mention of the bioethical considerations of this issue, as they constitute one of the ongoing interests of this subcommittee. We have looked at a large number of aspects of the genetic revolution.

Do you believe that we might reach a point where these technologies could lead to ethical breaches?

Dr. ZIMBELMAN. Do you mean applications to humans that are——

Mr. GORE. Well, certainly that would immediately raise bioethical questions, but at what point should we raise the red flag where animals are concerned? Never?

Dr. ZIMBELMAN. I guess I have not seen any proposals which I would be concerned about in that regard The way things change, I don't think you could say never.

Mr. GORE. You mention the concept of precedents. That is a difficult concept when you are talking about animals. I mean, I accept your answer. I have no reason to challenge it.

I am troubled at the reports that in another country there have been two reported alleged incidences of human embryos being frozen and then implanted and brought to term. Are you familiar with those incidences?

Dr. ZIMBELMAN. No.

Mr. GORE. Dr. Karihaloo?

Dr. KARIHALOO. Yes, but there was nothing very unusual about those.

Mr. GORE. Well, it depends on your definition of unusual, I guess.

Dr. KARIHALOO. The same way they collected the embryo and defrosted it until they had an appropriate recipient available.

Mr. GORE. Yes, but the bioethical questions are immense there.

Dr. Karihaloo, you were extensively quoted in the Business Week article of June 21. Among other things, the article had an illustration of a giant dairy cow that dwarfed its owner.

Was that cow contented?

Dr. KARIHALOO. By our definition, Carnation's definition, yes, she better be.

Mr. GORE Would you say that your optimistic projections were fairly characterized in that account or would you like to amend them or do you think that the revolution in dairy production is indeed relatively close?

Dr. KARIHALOO. Well, the same thing as we discussed all this morning, gene amplification and selective breeding, I wouldn't be very surprised, Mr. Chairman, not necessarily that we will have a cow looking like an elephant.

The idea was that in the future a cow, whether it is a beef or whether it is a dairy cow, will be very efficient in terms of utilizing the feed and analyzing the production, whether it is for beef—say, for example, an animal could go to slaughter at 4 months of age as against now 2 years. By the same token, a cow producing 30,000 pounds of milk could easily produce 60,000 with far less feed.

This has more application in developing countries where people don't have enough to eat and leave aside their animal's feeding. I certainly believe that is going to happen.

Mr. GORE. Dr. Zimbelman, do you want to comment on that?

Dr. ZIMBELMAN. We started working on bovine growth hormone in my unit a number of years ago when I was convinced that the technology of measuring hormones and other metabolic changes allowed us to understand growth better.

But there are still many questions we have today about whether growth hormone is the limiting factor in bovine growth and milk production and if there is real potential to manipulate it in a useful manner.

Now a breakthrough has come by getting E. coli to produce growth hormone, and we are joining that race. I guess I still have to be cautious about whether I think that a product will be successful.

There are a lot of challenges, particularly when you talk about those things impacting on the entire cattle business. If something is in hand today and pretty well proven, it is 3 to 5 years off getting it through the FDA because of all the toxicity and efficacy questions we have to answer and reanswer.

I guess that is where some of my concerns come from. I think there is great potential in the technologies discussed today. I think we need to consider it carefully, we need to do the right kind of research, but I think we can do ourselves harm in two ways. We can be too pessimistic and say it should be forgotten, it is not going to happen at all, or we can oversell and have people expect too much too soon and then becoming impatient with less progress than promised.

I hope I am trying to come out right in the middle and say it has got potential, and we need to keep going. If growth hormone expressed by E. coli happens to work and we find ways to deliver it, I don't mind at all taking advantage of those opportunities that come up before we have all the answers.

I think the only way to plan that coming about is to continue answering their basic questions and those questions that are important. So, I guess that our posture is to say there are some things coming here that we can't ignore, but let's not get oversold and impatient and write them off because we tried to hurry too much with them.

That is where I am coming from.

Mr. GORE. Both of your companies, I take it, see a pretty large market in these technologies at some point in the future. I take it that you would say the timeframe is a little bit longer than you would, Dr. Karihaloo, is that fair to say?

Dr. Karihaloo. This is a very difficult question to answer. I foresee a lot of changes, Mr. Chairman, in another 15 to 20 years, not necessarily a contented cow, as you described, but there will be a lot of steps toward that direction.

Even so, there will be a lot of changes in the technology we have at hand now. That needs to be improved, and there is intense work not only by the universities or the research organizations, but by the private companies, maybe only for their own profit.

Nevertheless, there is a lot of work being done toward achieving that goal. I am very optimistic, so to speak, that this technology will be applied in the very near future.

Mr. Gore. Dr. Zimbelman, is there a perception on your part or on the part of your company that this technology, although it is a very high risk R&D expenditure, is a means of hedging one's corporate bet and that if there are breakthroughs in the future of the company, without the technological capability it could fall hopelessly behind?

Dr. Zimbelman. I think that is what I tried to say in my testimony.

Mr. Gore. That is sort of your approach, then?

Dr. Zimbelman. I think that is why I don't dismiss it easily or be too pessimistic. If I felt that somebody was going to be on the market 5 years from now with cattle in which they have inserted a bovine growth hormone gene and therefore there would be no need for bovine growth hormone, then we ought to quit investing in that research today.

So, I guess by continuing to support our research I am saying it is a little further off than that, but I have to keep monitoring it because if things happen faster in that area, then it needs to impact on our decisions and possibly make changes.

Mr. Gore. Thank you very much for fascinating testimony. You have helped us triangulate on a little more accurate view of what is likely to occur. Thank you so much.

Our final witness today is Dr. Dan B. Laster, the National Research Program Leader for Beef Cattle and Sheep Production from the Agricultural Research Service, with USDA here in Washington.

It is nice to have you here, Dr. Laster. Welcome. Without objection, we will put your statement in the record in full.

I understand you have had some experience in Tennessee; is that correct?

Dr. Laster. Yes, sir. I am from Tennessee.

Mr. Gore. Where abouts are you from in Tennessee?

Dr. Laster. Henderson County, west of Carthage.

Mr. Gore. I know where Henderson County is. Well, that makes it a double pleasure to have you here then. I want to invite you to proceed. We will put your full statement in the record without objection and you can present all of it, or a summary, as you see fit.

STATEMENT OF DR. DAN B. LASTER, NATIONAL RESEARCH PROGRAM LEADER FOR BEEF CATTLE AND SHEEP PRODUCTION, AGRICULTURAL RESEARCH SERVICE, DEPARTMENT OF AGRICULTURE

Dr. LASTER. Thank you.

I am very pleased to have the opportunity to be here today and to discuss the status and potential impact of biotechnology on animal agriculture. My comments perhaps will be a bit broader than some of the other speakers earlier this morning.

First, I would like to comment on the state of the art in some of these areas. I think it is relevant to point out that our increases in food production in the last 30 to 40 years have come about in two ways: the development and implementation of technology and simply by increasing the amount of cropland acreage.

We have a finite amount of land. It is fixed. Second, we have a lot of land converted from pastures to farmland that is very marginal. We have land where we are growing soybeans and the yield continually goes down, particularly in the South.

So, if we look at increases in population within a reasonable timeframe, say 30 years, we expect to have increased pressure for the development of new technologies. New technology will become essentially our only option in this country to increase food production.

I would like to discuss the area of biotechnology from three standpoints or three areas:

The first one relates to the embryo transfer area, of which we had very excellent presentations this morning, and associated technologies such as cloning, sexing of sperm and in vitro fertilization and culture of germ cells.

The second and third areas are really aspects of genetic engineering. One is the use of recombinant DNA technology to produce vaccines and hormones or other protein regulators. The third one is the use of genetic engineering procedures to alter the genetic makeup of the whole animal for disease resistance or for other production traits.

These two areas I will comment on a bit later, but one is relatively simple; it is not simple, but the one using bacteria fungi or yeast in a fermentation system is relatively simple compared to altering the genetic makeup of the whole animal.

I think each of these three areas in biotechnology has the potential—and I want to emphasize potential in terms of total industry—of increasing biologic efficiency of the individual animal; that is, allowing an individual animal to convert more product, whatever the product may be, from less resources.

Second, I think that it gives us greater flexibility of fitting the animal germ plasm base that we have to our feed resource base, recognizing that the feed resource base is going to change over the next 50–100 years.

Embryo transfer is being used in the cattle industry today to a very limited extent, as you well know and as has been pointed out earlier today. There are basically two constraints in embryo transfer itself today in terms of limiting its application or making it more expensive because of the relative efficiency.

The first area relates to the production of a large and predictable number of ova or embryos, or superovulation. The second area is our inefficiency at the present time on long-term storage of embryos, or deep freezing. In each of these areas we certainly need more information; one, in the case of ovulation control, and two simply in cryopreservation.

Within that same area, I don't think we need to be concerned at this point in time that within the next 10 years cloning or genetic engineering is going to revolutionize the animal agricultural industry to the point it is going to make it hard for the small producer or any other.

I think our concern needs to be that within the next 30 to 40 years, will we have these technologies so that we can produce enough food. So, that is our concern, rather than the first part.

One of the questions that was raised by the Chair this morning related to, in terms of transfer and cloning, the effects on limiting the base of germ plasm. I believe it was by Congressman Walker.

I think the efficient use of embryo transfer and particularly storage will enhance it. It limits it on one side, but the big constraint we have today of introducing and maintaining animal germ plasm is disease, whether it is from Africa on other countries on a global basis.

So, disease control in transport of germ plasm within a country or among countries would be greatly enhanced. This would allow us to maintain a diversity of animal germ plasm on a national and global basis. Without it we cannot maintain our germ plasm base. We cannot maintain big zoos all over the world.

Mr. **Gore**. Let me interrupt you there. What you mean there is that you can't easily transfer genes between continents carried by live animals because of the quarantine requirement, but in the form of frozen embryos it would be easy to do so.

Dr. **Laster**. Yes, sir, that is right. I think that would enhance this side of the issue. Obviously, as was pointed out, if you can clone in these other areas, you are going to select the better animals and narrow your genetic base on the one side, but this would also allow you to preserve and broaden that base.

Mr. **Gore**. But the overall diversity on both sides of the ocean would be narrowed, wouldn't it?

Dr. **Laster**. It would be narrowed, but you could store what you presently have, too, in terms of a germ plasm bank on a global basis.

Mr. **Gore**. How are we going to fund that?

Dr. **Laster**. It has been our suggestion it would be handled by FAO, Food and Agriculture Organization of the U.N., which is the logical source to do it.

Mr. **Gore**. Has there been a serious proposal of that kind?

Dr. **Laster**. There has been a study and a report looking at how we can maintain genetic diversity. The real problem at this time relates to the inefficiencies in deep freezing of embryos. If we had it more efficient and more cost effective, then at least that would provide the avenue.

Mr. **Gore**. Well, that proposal implicitly shares the concern expressed by Congressman Walker, if there is a proposed fix for that concern.

Dr. LASTER. Yes, sir, that is right.

Mr. GORE. Go ahead.

Dr. LASTER. Cloning to me, regardless of definition, implies large numbers of the same genetic material. I think the definition I agree with, but when we say identical twins, normally that does not infer cloning, although by a straight definition it may be.

But at least in beef cattle we have had splitting of blastocysts to get either up to a maximum of three or four animals. In other words, we are a long way from being able to clone these 100 animals to do what you have pointed out to the previous two groups. At least I think we are.

In vitro fertilization has been demonstrated in some species, but this and in vitro maturation of germ cells I think is some time away from implementation by the livestock industry.

Sexing of embryos, as has been pointed out—embryos can be sexed today, but it still is relatively inefficient. It is our opinion, based on research results, that we cannot accurately sex sperm today.

It appears that both efficient and effective methods for sexing semen could be developed if we focus on a NASA-type approach with appropriately trained scientists from the different disciplines needed. With this as a target, it could be accomplished in a reasonable time frame. I am not sure about the exact number of years, these are relatively long-term and high-risk areas, but at least it would seem feasible.

An important factor that I think we should recognize that has been pointed out very well, and I think it is important, is that development of genetic engineering as it relates to the whole animal, is dependent upon manipulation of embryos and storage. In other words, we have to have efficent embro production, transfer and storage techniques before we can really even do research in whole-animal genetic engineering areas.

The other thing that I would like to call attention to is that these areas of embryo transfer, used broadly, but transfer, cloning, sexing of semen, in vitro fertilization would be highly synergistic. In other words, if we could effectively clone embryos, this would enhance the use of transfer and storage.

I think it is obvious, but in terms of voids of the information, we have some voids within this broad area that is need attention before we have broad use of the techniques in the livestock industry.

The second area, of which I am well aware a month ago in the plant area was covered for you, is the use of genetic engineering or recombinant DNA for the production of vaccines and hormones.

I won't say very much about that other than the development of the foot and mouth vaccine, the production of growth hormone and insulin it would seem to me at this point simply provide a small window for us to look at the potential in terms of the true effectiveness of the implementation and the efficiency of the production.

We think we need a lot of research in those areas before they can really be used commercially. I think it is important that in the use of the recombinant DNA technology for the production of growth hormone, we are dealing with a protein where we knew the chemical structure of growth hormone in the early 1960's, 1961 or 1962.

We have this technology and we produce it, yet we perceive of using that to produce all kinds of things for which we do not know the structure. Not that it isn't possible, it is just that we have to have those kinds of understandings before we can do very much with recombinant DNA techniques.

Lastly and briefly, a comment on genetic engineering as it relates to changing composition. I think we have three constraints in this area.

The first one is the complexity of the system. I don't think these problems are insurmountable, but they are real. Most economic traits we think are controlled by more than one set of genes and gene interaction.

The second constraint is we don't know much about it, the structure, the organization, the mechanisms.

The third one, it is my opinion that we do not have an appropriate number of well-trained people in these areas of molecular biology to focus in this area. It is no different in this area than it is in the fact that when we breed a beef cow to a bull we get about 65 to 70 percent pregnant. If we breed AI we get something slightly less than that at one estrus.

In many areas of animal agriculture, we simply do not have the necessary understandings of the biological processes to develop the technology. I think Dr. Zimbelman pointed this out quite well in his statement. It is true in each of these areas, although some are further developed than others.

It seems to me that to this point the public support for basic research and industry supported, product development has worked quite well. I think it is imperative that we continue this type of approach in these areas and not assume that these biotechnologies are developed or going to be developed in the near future on a highly efficient basis.

The Agricultural Research Service has some resources committed to these areas, as we have discussed today, and has plans to increase resources in some of these areas in the future to the standpoint that it is feasible relative to other societal needs and I suppose pressures for specific needs.

It is our strong opinion that we need to strengthen the ties between agricultural research and the other areas of biological research. As we do this, we will be in a much better position to protect our continued food supply.

I thank you, sir.

[The prepared statement of Dr. Laster follows:]

Prepared Statement of Dr. D. B. Laster

Mr. Chairman and Members of the Subcommittee, I am pleased to have the opportunity to discuss with you today the status and potential role of biotechnology in animal agriculture.

The food and agriculture system in the United States accounts for about 20 percent of our nation's gross national product with the animal component providing about one-half of the total. Increases in food and fiber production to meet the U.S. and world needs have been achieved by implementation of new production technologies and by increasing cropland acreage. Due to soil erosion, diminishing water supplies for irrigation, the finite amount of land suitable for crop production and the need to convert marginal lands from crops back to forages, the pressure for development of technologies to increase biological efficiency of animal and plant production will likely increase substantially during the next two to three decades.

We are here today to discuss some of the areas of science and technology that have the potential for making quantum increases in the biological efficiency of animal production.

I would like to divide the topic of biotechnology in animal agriculture into three areas. The first area includes technologies for manipulation of gametes and embryos in the normal process of reproduction. This is the area of embryo transfer, gamete and embryo storage, cloning and splitting of blastocysts early stage embryos, sexing of sperm and embryos, in vitro fertilization and in vitro maturation of germ cells. The second area is the use of genetic engineering, recombinant DNA technology, or whatever terminology you prefer, as a means for production of vaccines, hormones and other protein regulators of biological processes. The third area is the use of genetic engineering procedures to permanently alter the genetic makeup of animals to increase genetic resistance to disease and increase productivity for traits that are controlled by quantitative gene expression; such as, reproduction, growth and composition. Obviously, the latter two categories are simply divisions of genetic engineering based on the complexity of the genetic systems into which the desired genes are to be incorporated to deliver the desired product or functions. The one area involves a relatively simple genetic system such as bacteria, yeast or fungi and the second a much more complex whole-animal mammalian or avian genetic system.

Stated in general terms the implementation of these biotechnologies in animal agriculture will increase the rate of genetic improvement, increase the biological efficiency of the individual animal so that the animal will produce more product on less resources, and provide greater flexibility and opportunities to fit animal germ plasm resources to the different and dynamic productive ecosystems.

Embryo transfer is being used in the cattle industry today primarily to increase the reproductive rate of valuable cows; for example, cows that have or are perceived to have a high genetic worth for the desired traits. Embryo transfer has numerous other valuable uses in the livestock industry. This technology will be utilized more widely in the livestock industry as it becomes more efficient. An important constraint affecting efficiency of embryo transfer is the production of a large and predictable number of viable ova and transferable embryos. Thus, there is a need for more basic endocrinology and immunology research to develop the necessary understanding for control of gamete production.

One of the major constraints for exploitation of embryo transfer is the inefficiency of present procedures for long-term storage of embryos. Although the deep-freezing of cattle embryos is being used to a very limited extent in the purebred cattle industry, the technology is too inefficient for widespread commercial use by the livestock industry. Voids in our understanding of cryopreservation of mammalian tissue are constraints on faster and more efficient technology development in this area.

Embryo transfer and storage technologies provide excellent means for increasing animal productivity through several avenues. Some of these are by increasing the rate of genetic improvement, more efficient progeny testing of potentially highly valuable animals, disease control in transport of germ plasm within a country and among countries of the world, maintenance of diversity of animal germ plasm on a national or global basis, and a means to produce twinning in beef cattle. Presently, at least three public institutions, the Agricultural Research Service and the government supported research organizations of Australia and France, are selecting cattle populations for twinning. When the twinning rate of these populations is sufficiently high to be effectively used by the beef cattle industry, the most effective means to utilize this germ plasm commercially is through efficient embryo transfer and storage programs. Presently, embryo transfer is also being used as an important research tool for studying embryonic mortality and maternal effects on embryo and fetal development.

Cloning, or at least the splitting of blastocysts has been demonstrated in cattle, but needs considerable research and development before the technology is commercially feasible in the livestock industry. In vitro fertilization has been demonstrated in some species, but this technology and the in vitro maturation of germ cells from farm animals appears to be some time away from livestock industry application.

Sexing of embryos can be accomplished at a somewhat inefficient level, but sexing of sperm cannot be done with any reasonable level of accuracy at this time. It appears that efficient and accurate sexing of sperm could be accomplished by a multi-institutional effort involving a team with members representing the disciplines of immunology, cytogenetics, physiology, biochemistry, electrical engineering and mathemalar biology. Such an effort would be long-term and high risk and the relative impact should be carefully assessed before such a venture is initiated.

An important fact we must not overlook is that most any research and development in the genetic engineering field and the implementation of genetic engineering technology will require efficient procedures for in vitro culture and manipulation of embryos and embryo transfer and storage procedures, Thus, technologies and methodologies for embryo manipulation are essential components of whole-animal genetic engineering research and development.

Each component of the gamete and embryo manipulation area is highly complementary to commercial implementation of the other areas, Development of efficient technological procedures for long-term storage of embryos, cloning, sexing of sperm and/or embryos, and in vitro fertilization in some species will have a synergistic effect on implementation of the related technologies.

I would now like to comment on the use of genetic engineering for the production of vaccines, hormones and other proteins with important biological regulatory function. This is an area where we presently have examples of the use of genetically engineered microorganisms to synthesize proteins of clinical importance and mammalian tissue products such as insulin, growth hormone and interferon by use of recombinant DNA procedures.

The production of a genetically engineered vaccine against the foot-and-mouth viral disease represented a breakthrough in the use of recombinant DNA technology. It should be noted that over two decades of concentrated research effort formed the basis for this development prior to the advent of recombinant DNA technology. Vacciees that are safe and economical can be produced by recombinant DNA technology and with this procedure the dangers of spreading the disease by exposure of animals to a live virus are eliminated.

Development of the vaccine for foot-and-mouth disease and the production of growth hormone, insulin and interferon through genetic engineering represent breakthroughs in efficient and effective methods for production of important proteins. These technology breakthroughs provide an effective yet small window for viewing the potential for the impact of genetic engineering on animal and plant production.

I am confident that through further research and development there will be many other genetically engineered vaccines and physiological products produced in a similar manner. Many of these products will likely be beneficial to animal agriculture. One area with a relatively high probability of success would seem to be the microorganisms involved in rumen fermentation. This area is somewhat an interface between the microorganism and whole-animal genetic system.

It is important to remember that in this area of genetic engineering we are dealing with bacteria, yeast, or fungi in the fermentation system where much is known about the genes and biochemistry of the system being manipulated. Additionally, the structure of insulin and growth hormone, for example, were determined several years ago and each of these hormones had been synthesized long before attempts were made to produce them by recombinant DNA techniques.

Next I want to discuss the use of genetic engineering to change the genetic composition of animals to increase genetic resistance to disease and to alter other production traits.

There are several, but not insurmountable, constraints associated with this area. First is the complexity of the myriad of genes in any animal and the fact that most traits of economic importance in farm animals are apparently controlled by many genes. In most instances we think the genetic control is elicited by many genes and gene interactions. A major constraint in this area is our lack of understanding of the structure, organization, and mechanism of expression of the basic genetic systems of a mammalian specie. In addition, it so often difficult to separate the genetic component of expression for the environment component because of genotype-environment interactions. The real problem for development of genetic engineeering and other new technologies in many areas of animal agriculture is that the basic biological data base is running low relative to the technology development and obvious opportunities for technology development. In many areas of animal agriculture we imply do not have the necessary understandings of the biological processes to develop the technologies needed for increasing biological efficiency of animal production. This is true for increasing the effectiveness and efficiency of superovulation for embryo transfer and cryopreservation of embryos as well as for the use of genetic engineering procedures for altering the genetic makeup of the whole-animal.

The third constraint for development of genetic engineering technologies for use in the whole-animal is the availability of scientists appropriately trained in microbiology, immunology, genetics, and physiological-genetics, endocrinology, biochemistry, physiology, and other areas of molecular biology to focus on the basic areas of inheritance factors and physiological mechanisms to develop the necessary biological understandings for technology development. There is a shortage today and I believe there is need for concern that Federal research and acedemic research and teaching institutions committed to long-term basic research may not be able to compete with private industry which is more concerned with short-term "payoffs" for talented scientists in these areas of reseach and development.

I believe we already have enough evidence in the embryo manipulation and genetic engineering areas to indicate that these technologies have a tremendous potential for increasing food and fiber production both totally and in more efficient and effective ways than our present technology.

I firmly believe that genetic engineering technology has tremendous potential for increasing productivity of animal agriculture. But it concerns me when I read articles in daily newspapers and popular magazines that due to genetic engineering technology the future beef cow will be able to produce an average of over 5,000 pounds of beef per year rather then the present 800 pounds on about the same amount of feed with the implication that this technology is just around the corner. I think such projections are achievable, but the scientific community must begin making a much higher commitment to research focused on these targets if this technology is to be available within the next 30 years.

The combination of public supported basic research and industry supported research for product development has brought us to where we are today in the areas of embryo manipulation and genetic engineering. I think it is imperative that we continue this same approach in areas of opportunity for continued development of new technologies. Development of these type technologies is essential for sustaining the security of our food and fiber production systems.

The Agricultrual Research Service has some resources committed to the areas we have discussed today and has plans to increase resources in these areas. It is our plan to commit a greater proportion of resources to research programs that are relatively long-term and high risk. It is my opinion that the entire publicly supported agricultural research community is supportive of these as the areas of basic research that will be fruitful and will commit as many resources to them as possible. This commitment will help to strengthen the bonds between targeted agricultural research and other areas of biological science of which agricultrual research must become an increasingly important part if we are to develop the essential food and fiber production technologies that will be essential in the future.

Mr. Chairman, this concludes my prepared statement. I will be happy to respond to any questions you may have.

Mr. GORE. Thank you very much. Excellent testimony.

What is the Federal budget for genetic applications to animal husbandry? Could you estimate it?

Dr. LASTER. I am not real sure. I simply don't know. I am sorry. We can get it provided to you in terms of what is biotechnology or genetic engineering, but I simply don't know.

Mr. GORE. You can provide that for the record. I would appreciate it.

Dr. LASTER. Yes, sir.

[The information follows:]

In fiscal year 1982 for genetic engineering in animals, ARS budget was $2,485,714 and 9 scientist years; for genetic engineering in plants, ARS budget was $2,364.381 and 14.2 scientist years—total ARS budget for genetic engineering in fiscal year 1982 was $4,850,095 and 23.2 scientist years.

Mr. GORE. Is it your view that we were not doing as much as we should be doing?

Dr. LASTER. Yes, it is my view. We are making an effort and have plans to increase a reasonable proportion of work to this area.

Mr. GORE. Is USDA undertaking any research in the cryopreservation area?

Dr. LASTER. A very slight amount as far as animals are concerned, and I am simply not familiar with the plant area. We have some work in terms of freezing of embryos. It tends to be more for use of studying biological processes than in the real research or real area of cryopreservation.

Mr. **Gore**. Because of the synergy you referred to earlier?

Dr. **Laster**. Yes, sir.

Mr. **Gore**. How is the work of the Department transferred to the private sector?

Dr. **Laster**. Well, a number of ways. Are you talking about technology transfer? I am part of ARS. We have a Federal extension service. From a Federal standpoint——

Mr. **Gore**. Do they get involved in this sort of business? This is still a little premature for ARS, isn't it?

Dr. **Laster**. Are you talking about from the area of transferring what we have or simply the——

Mr. **Gore**. Yes.

Dr. **Laster**. Yes, they do, and I would say most of the people who testified here this week, I and other scientists in ARS, and we will see them week after next in a scientific meeting. It is a relatively small area if we look at reproductive biology, even.

Mr. **Gore**. Yes.

Dr. **Laster**. So the contacts from industry to State and Federal is really very high. Now, that is simply exchange of information and research planning. It may not be the point you mean.

Mr. **Gore**. Well, I am impressed that the USDA's performance in this field is as good as everyone that we have talked to says it is. I am encouraged by that. As I said in my opening statement, it is not always that way, but I guess that is a tribute to your work and those who work with you. I am interested in the best possible ways to disseminate the work done at USDA to the private sector.

You have identified the scientific manpower problem in your statement. This was something that we encountered in an earlier hearing on plant biotechnology. Do you or does the Department have any proposals for alleviating this problem of scientific manpower?

Dr. **Laster**. By manpower we are talking about appropriately trained people.

Mr. **Gore**. Right.

Dr. **Laster**. If we do, I am not aware of it. I simply do not know. I think it is a concern expressed by a number of people, a concern relating to where is the training to come from. In the past it has come through other avenues, or other areas have added to the agricultural sector, and it seems less likely to continue in that direction over the next 20 years.

Mr. **Gore**. I would be interested if you came up with any ideas on that. The subcommittee has been concerned about that for quite some time.

Dr. **Laster**. We will see if we can provide that, if there is a plan. I am not sure.

Mr. **Gore**. All right.

[The information follows:]

There are few current Federal initiatives to stimulate the development of animal molecular geneticists. While there are no present fellowship or grant programs administered by the U.S. Department of Agriculture to stimulate graduate student enrollment, some very limited indirect support is provided to graduate students majoring in animal molecular genetics in the form of graduate research assistantships. It is estimated that fewer than fifteen percent of the agricultural graduate students enrolled in animal molecular genetics programs are recipients of research assistantships as a result of Federal research appropriations.

Mr. **Gore**. I want to thank you for coming here today, and I want to thank all of our witnesses. I have found it a fascinating hearing, and I think as a result of the testimony today we can prepare a report that will have a much more accurate forecast of this technology's impact than the preconceptions I had going into the hearing. It has been very, very interesting for me.

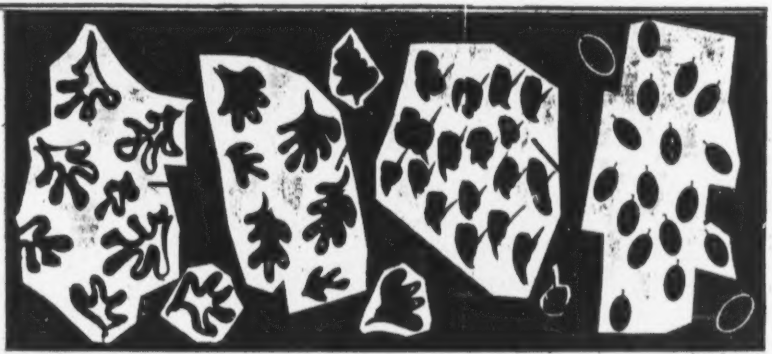
I want to again congratulate you on the work you have done and thank all of our witnesses for their help today.

We stand adjourned.

[Whereupon, at 1:42 p.m. the subcommittee adjourned.]

Green Revolution II: $

By Jack Doyle



Laurie Rosenwald

WASHINGTON — There is a new Green Revolution coming to America, and its promises are many and high-sounding. We are told that a dazzling new cornucopia of miracle crops and genetically engineered substances will set farmers free of expensive fertilizers, yield cheap and abundant foods, and enable the United States to feed the world's hungry. Last month, Forbes magazine proclaimed "Green Revolution II" the growth industry of the 1980's. Indeed, this new agri-genetic revolution has turned the head of Wall Street and big business.

During the last 10 years, 20 "Fortune 500" corporations have acquired at least 60 United States seed companies. The Allied Chemical Corporation, Standard Oil Company of California, and National Distillers & Chemical Corporation are a few of the corporations investing in new genetic-engineering firms, where everything from manipulating wheat genes to cloning potato plants is under way. The Monsanto Company has a $20 million stake in Biogen, N.V., a Swiss firm, and has also invested in Genentech Inc. and Genex Corporation. Monsanto and Genentech recently developed a new "bovine growth hormone" — a genetically produced substance that could be injected into dairy cows and beef cattle to increase yields.

E. I. DuPont de Nemours & Company, Dow Chemical Company, and Allied — corporations that sell chemical fertilizers to farmers — have been working on nitrogen fixation, a process that could make major crops independent of fertilizers. Dow has also been researching photosynthesis, Pfizer Inc. has invested several million dollars in plant genetics, and the Union Carbide Corporation is involved in plant hormones and bio-regulation. When the chemical-replacing substances of genetic engineering burst onto the market, these corporations will have "a piece of the action."

Unlike Green Revolution I, the new agri-genetic revolution will not necessarily give farmers more control over production. The new superproducts manufactured by the DuPonts and the Monsantos may only be affordable by the 3 percent of farmers who now account for 40 percent of all sales of farm goods. Further, the biological instructions lodged in new seed or genetically improved agricultural substances could require farmers to make expensive capital improvements to stay in business. Over time, the largest of America's farms could come under contract to the laboratory-based corporations, using corporate-bred plants, chemicals, and hormones. Moreover, given the pivotal role of the seed and gene in transmitting biological and economic values, food crops might one day be ordered and formulated in the lab, especially when large international trading companies want a certain kind of grain in supertanker proportions.

In an era of booming agricultural exports, food quality and genetic diversity might not fare well under the corporate mantle. Volume and durability may matter more. Plant-breeding programs have not always made nutrition their highest order of business. Such traits as color, harvestability, plant sturdiness, ability to travel well and ripen "on time" tend to show up more regularly than nutrition. Genetic diversity and disease resistance are a bit more difficult to achieve: A devastating corn blight visited the United States in 1970, despite "stronger" hybrids. January's Scientific American warned that "the range of genetic variation of the cultivated wheats has decreased drastically in recent years." A new "time bomb" is ticking. Yet, a boundless, almost blind faith in a technological fix for agriculture exists throughout the land.

So, as we enter this new era of the cloned potato and corporate-directed farming, who will speak for nutrition, genetic diversity, and competitive pricing? And does it matter who owns and controls our food supply? While Congress is currently mired in its quadrennial farm-bill fight — attending essentially to "maintenance" questions — the most important "structural" questions of farm and food policy are rarely asked.

Pretending that the corporate-investment pattern in today's "brave new agriculture" does not exist, or does not pose real concerns about the quality and control of our food supply, will only make the day of reckoning all that more difficult. The historical lessons of malfeasance in pesticides, toxic wastes, farm-worker health and safety, and carcinogenic food additives should be all too fresh in memory to allow a new, more-insidious form of corporate dominance to emerge. This time though, the stakes are higher. Genetic "spills" might become unmanageable; crop blights and pestilence might become more prevalent; and the costs of new "superfoods" could have wide-ranging farm and social consequences.

Not to ask hard questions now would be to turn over the nation's sustenance to the Monsantos and Dows of this world.

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